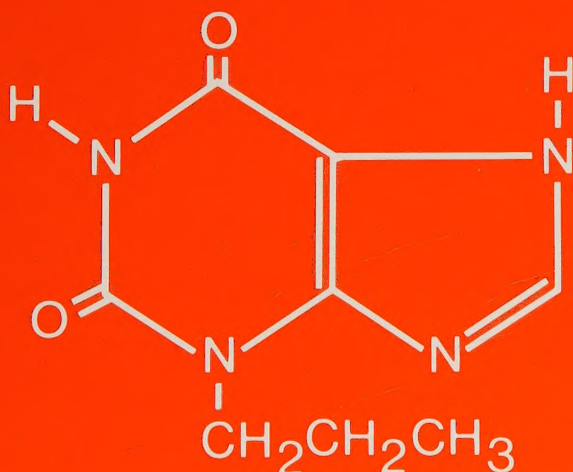
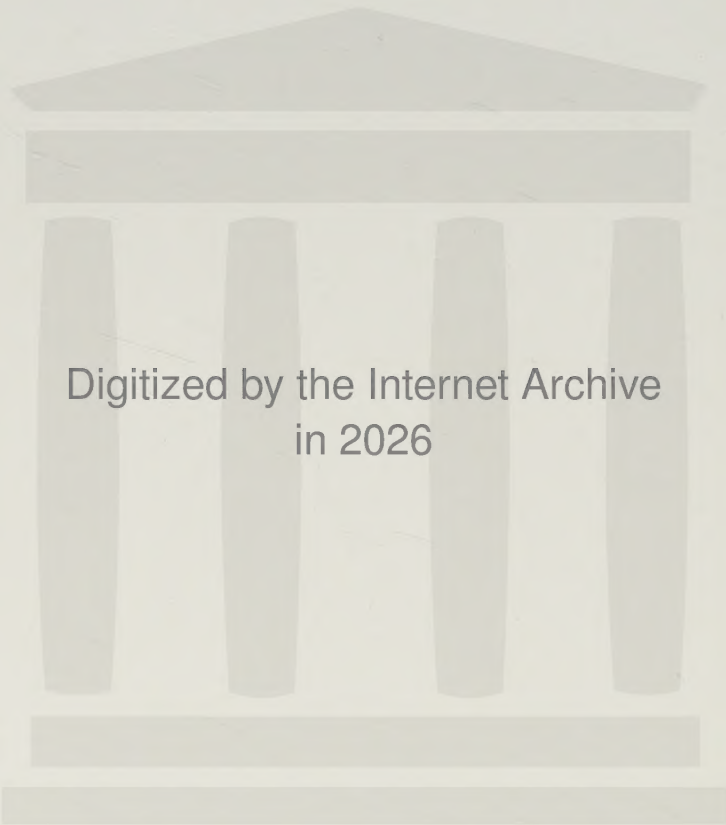


On the
Pharmacodynamics and Pharmacokinetics
of Enprofylline
– a New Antiasthmatic Xanthine

by
NILS JOHANNESSON



Lund 1983



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ON THE PHARMACODYNAMICS AND PHARMACOKINETICS OF ENPROFYLLINE - A NEW ANTI-ASTHMATIC XANTHINE.

Abstract

It has recently been proposed that antagonism of endogenous adenosine at receptor levels may explain the broad spectrum of effects exerted by xanthines. To investigate the importance in man of this hypothesis comparative studies between a potent adenosine-blocking xanthine, theophylline, and a non-blocking xanthine, enprofylline, have been performed.

Two studies in asthmatic patients revealed that enprofylline is a potent bronchodilator at plasma concentrations consistent with a potency ratio of five to one compared to theophylline. Both enprofylline and theophylline, at a plasma concentration ratio of one to four, induced a slight protection against immediate allergen induced bronchoconstriction while both xanthines protected against late allergen induced bronchoconstriction.

Three studies, each involving eight healthy volunteers, showed that, at a plasma concentration ratio of five to one, theophylline and enprofylline have similar effects on the lower esophageal sphincter pressure (LESP) while theophylline, but not enprofylline, induce natriuresis, release of free fatty acids (FFA), and stimulation of gastric secretion.

The results show that bronchodilatation, effects on allergen induced bronchoconstriction, and relaxation of LESP by xanthines are mediated by mechanisms unrelated to adenosine receptor antagonism, while natriuresis, FFA-release, and stimulation of gastric secretion may be related to this mechanism.

Enprofylline undergoes negligible metabolism in man and has a high renal clearance. A study in six asthmatic patients revealed similar distribution kinetics for enprofylline and theophylline. The absorption of enprofylline from the stomach, duodenum, and colon was also investigated in six healthy subjects. While a good and reproducible absorption was seen from the intestines enprofylline was poorly absorbed from the stomach.

Key words

enprofylline, theophylline, adenosine receptor antagonism, pharmacokinetics, xanthines.

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Date 1983-10-18

From the Department of Clinical Pharmacology
University of Lund, Sweden

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Lund 1983



To my beloved wife, Anita

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 Rahms i Lund

PREFACE

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals:

- I. Enprofylline - Effects of a New Bronchodilating Xanthine Derivative in Asthmatic Patients. L.C. Laursen, N. Johannesson, A. Dirksen, R. Djurup, E.P. Munch, E. Taudorf and B. Weeke. Allergy 38:75-79, 1983.
- II. Intravenous Administration of Enprofylline to Asthmatic Patients. L.C. Laursen, N. Johannesson, P.-O. Fagerström and B. Weeke. European Journal of Clinical Pharmacology 24:323-327, 1983.
- III. The Effect of Theophylline and Enprofylline on Allergen Induced Bronchoconstriction. R. Pauwels, D. Van Renterghem, M. Van Der Straeten, N. Johannesson and C.G.A. Persson. Submitted for publication.
- IV. Increase of Plasma Free Fatty Acids and Natriuresis by Xanthines may reflect Adenosine Antagonism. K.-E. Andersson, N. Johannesson, B. Karlberg and C.G.A. Persson. European Journal of Clinical Pharmacology. Accepted for publication.
- V. Relaxation of lower esophageal sphincter, and stimulation of gastric secretion and diuresis by antiasthmatic xanthines. Role of adenosine antagonism. N. Johannesson, K.-E. Andersson, B. Joelsson and C.G.A. Persson. Submitted for publication.
- VI. Absorption of Enprofylline from the Gastrointestinal Tract in Healthy Subjects. E. Lunell, K.-E. Andersson, O. Borgå, P.-O. Fagerström, N. Johannesson, G. Kjellin, C.G.A. Persson and K. Sjölund. Submitted for publication.

<u>CONTENTS</u>	<u>Page</u>
INTRODUCTION	1
LUNG ACTIONS	3
RENAL ACTIONS	8
METABOLIC EFFECTS	10
GASTROINTESTINAL EFFECTS	13
PHARMACOKINETICS	15
GENERAL DISCUSSION	19
Pharmacodynamics	19
Pharmacokinetics	20
The future clinical value of enprofylline	21
ACKNOWLEDGEMENTS	23
REFERENCES	24
ENCLOSURES I-VI	

INTRODUCTION

Xanthines are today widely used as stimulants and as remedies against various diseases. Caffeine (1,3,7-trimethyl-xanthine) is a psychotropic agent that is contained in coffee and tea and a significant clinical application of theophylline (1,3-dimethyl-xanthine) is based on its antiasthmatic properties. Theophylline is also employed as a diuretic in the treatment of acute heart failure. The respiratory stimulant properties of both theophylline and caffeine are applied against apnea in neonates.

Besides beneficial effects the xanthines are known to cause adverse reactions such as cardiac arrhythmias, massive CNS-stimulation, nausea, vomiting, headache and gastrointestinal disturbances (for a review see ref.1). The risk of inducing these adverse reactions has always been a problem in the clinical application of xanthines. A complicating factor is the intra- and inter-individual variations of the liver metabolism of caffeine and theophylline (2) and a lot of work has been done to achieve a better understanding of the basic pharmacokinetics of xanthines.

Many hypothesis concerning the cellular mode of action of xanthines have been put forward in order to explain their various effects. Inhibition of phosphodiesterase by xanthines has since its introduction (3,4) been an often sited mechanism. This is, however, at present a widely criticized concept because the concentrations of xanthines necessary for obtaining

phosphodiesterase inhibition are well above the threshold concentrations for their pharmacological effects (5,6). A lot of interest has recently been focused on the fact that both caffeine and theophylline are potent adenosine receptor antagonists at clinically relevant concentrations (7,8,9). As a matter of fact caffeine and theophylline universally antagonise the effects of adenosine which has lead to the hypothesis that all clinically relevant actions of xanthines can be explained on the basis of this mechanism (10). This hypothesis implicitly assumes that adenosine is an important modulator of the various biological processes that are influenced by xanthines. As the role of adenosine in man is unclear it is still uncertain to what extent this mechanism explains all clinical actions of xanthines.

Enprofylline (3-propyl xanthine) is a novel antiasthmatic xanthine (11) which has been shown to have a negligible ability to antagonise the effects of adenosine in various tissues such as smooth muscle (12), heart (13), nervous (12) and adipose tissues (14) and thrombocytes (15).

It has been suggested (12) that by comparing the pharmacodynamics of enprofylline and theophylline in man one would be able to investigate the relevance of adenosine receptor antagonism as an explanation of the effects of xanthines. This idea has been applied in this thesis to discuss the antiasthmatic renal, gastrointestinal, and metabolic effects of xanthines in man.

Enprofylline has also been shown to undergo negligible metabolism and be excreted renally as unchanged drug (11,16). This could in principle mean less inter- and intra-individual

variations of clearance of enprofylline as compared to theophylline. To elucidate this item some aspects of the basic pharmacokinetics of enprofylline in man have been studied.

The obtained pharmacodynamic and pharmacokinetic information also allows some conclusions to be drawn concerning the future clinical value of enprofylline in the treatment of obstructive lung disease.

The antiasthmatic effects are treated in papers I, II and III while the renal actions are discussed in papers IV and V. Papers IV and V are also dealing with the metabolic and gastrointestinal effects respectively and the pharmacokinetic properties of enprofylline are investigated in papers I, II and VI.

LUNG ACTIONS

Since the work by Herman et al (17) theophylline has been established as a valuable antiasthmatic drug. The investigations by Jenne et al (2) have lead to a better understanding of dose-effect relations for theophylline and guidelines for dose-adjustment was established. Ellis et al (18) pointed out that children require different dosage regimens due to a higher degree of metabolism and Ogilvie et al (19) gave detailed descriptions how to handle the administration of theophylline taking into account patients with an atypical metabolism of theophylline. The concomitant introduction of various SR-formulations (20) suitable for an adequate control

of plasma levels has lead to a marked increase in the use of theophylline and it is today the most widely used anti-asthmatic drug.

Theophylline relaxes in vitro airway smooth muscle (21,22) and is a potent bronchodilator in man (23). It has been shown to inhibit histamine and metacholine induced bronchospasm (24,25) and to prevent exercise induced asthma (26,27). Theophylline increases mucociliary clearance in animal and man (28,29).

While theophylline has been shown to possess antiallergic properties in animal experiments (30) results concerning its effects on allergen induced immediate bronchospasm in man are varying (31,32). No data exist concerning effects on the late allergen induced bronchospasm.

It is worth while to consider the fact that theophylline is a potent adenosine antagonist and that adenosine could be a mediator of asthmatic symptoms (10,33). In support of this hypothesis, inhalation of adenosine by asthmatic patients, but not by healthy subjects, induced significant bronchodilation (34). Adenosine has moreover been shown to potentiate antigen induced mediator release from rat mast cells and human mast cells (33,35). Furthermore theophylline, at low and therapeutic concentrations, inhibits the in vitro effects of adenosine (10). All this information suggests a role for adenosine as an asthmamediator and that xanthines may exert their antiasthmatic effects via adenosine receptor antagonism.

These ideas are, however, not in accordance with in vitro and animal experiments that have demonstrated that enprofylline is about five times more potent than theophylline

in its bronchodilating and antiallergic activities (11,30). Three studies each involving eight patients have revealed that a potency relation of roughly one to five also is applicable in bronchorelaxation of obstructive lung disease (36,37,38).

Papers I and II are dealing with the bronchodilating activity of enprofylline in an independant set of asthmatic patients. The investigations have been performed as acute studies involving patients with reversible (more than 20% increase in $FEV_{1.0}$ 5 minutes after inhalation of 0.5 mg terbutaline sulphate) obstructive lung disease. The trials were performed as acute studies and in order to control the day to day variation of lung function a stability criterion based on the morning values E_i $i=1, \dots, n$ (n =number of investigation days) has been applied. After completion of the n days stability was checked by forming the mean value

$$M = \frac{1}{n} \sum_{i=1}^n E_i$$

and demanding that

$$\text{Max } \frac{|E_i - M|}{M} \leq 15\%$$

$$i=1, \dots, n$$

If this was not fulfilled the patients were asked to visit the clinic an extra day when E_{n+1} was measured and the

stability criterion applied by excluding one of the E_i 's $i=1, \dots, n$ and including E_{n+1} . If the stability criterion was fulfilled the odd day was repeated.

The results concerning stability of lung function in paper I ($n=3$; i.e. placebo versus two oral doses of plain tablets of enprofylline) and paper II ($n=2$; intravenous infusion of enprofylline or theophylline) suggest that this procedure should be used more frequently to control the stability of lung function.

The use of plain tablets, in paper I, had the advantage of allowing for a simple administration and as 30 patients were included it was practical to use tablets. However, monitoring of plasma levels of enprofylline as function of time after administration revealed an irregular absorption from the gastrointestinal tract resulting in broad distributions of time to peak in plasma concentrations. An analysis of mean values of spirometry ($FEV_{1.0}$) as function of time revealed significant effects of both doses of enprofylline relative to placebo while no separation of the effects of the two doses of enprofylline was found. A tendency for dose related effects was instead obtained by examination of the mean maximum of $FEV_{1.0}$.

In paper II the xanthines were administered intravenously to six asthmatic patients by one hour loading infusion and one hour maintenance infusion. In order to obtain the desired plasma levels ($5 \mu\text{g/ml}$ for enprofylline and $15 \mu\text{g/ml}$ for theophylline) the individual pharmacokinetic parameters were predetermined on two separate days when plasma levels were

monitored after infusion of 1.5 and 5.0 mg/kg of enprofylline and theophylline, respectively. By assuming linear kinetics the basic parameters were determined in a model independent way and infusion rates could be calculated.

Both xathines induced a similar degree of bronchorelaxation. The small number of patients exclude any detailed comparison between the two drugs. However, a calculation of effects on spirometry per unit of drug in plasma revealed a potency ratio of one to five in agreement with preclinical results (11,12). Hence, papers I and II confirm that enprofylline is a bronchodilator in asthmatic patients roughly five times more potent than theophylline.

The effects of enprofylline and theophylline on immediate and late allergen induced bronchospasm were studied in paper III in nine patients suffering from allergic asthma. The patients were included during a period of no symptoms and had to have a lung function within 80% of predicted normal value. The trial was performed over three different days. Placebo was always given first and enprofylline and theophylline on day two and three with randomized, double-blind, cross-over technique. The drugs were given by one hour loading infusion and six hours maintenance infusion. The obtained mean steady state concentrations of enprofylline and theophylline were 2.6 and 10 $\mu\text{g/ml}$, respectively. At the end of the loading infusion the patients were provoked by an allergen dose known to induce at least 20% drop in $\text{FEV}_{1.0}$.

Both xanthines had similar effects with slight but significant effects on immediate reaction and a complete protection

against the late reaction. The slight effect of theophylline on the immediate reaction is in agreement with the varying results that have been reported. The effect on late reaction has not previously been described and deserves further investigations. Relevant questions to address are whether it is possible to obtain the same results if the placebo treatment is completely randomized with the active treatments, if the results are modified when the infusion regimens are altered, and whether these results have any clinical significance.

The fact that a non-blocking xanthine is a potent anti-asthmatic substance seriously questions the role of adenosine as a mediator in obstructive lung disease. The bronchoconstriction observed after inhalation of adenosine (34) may be unrelated to the actions of endogenous adenosine and instead be a result of an unspecific hyperreactivity. As moreover both relaxant and constrictor effects have been demonstrated in isolated airway smooth muscle preparations (39) and that adenosine at higher concentrations inhibits mediator release from inflammatory cells (35) it is difficult to see a clear role for adenosine in obstructive lung disease.

As a corollary, it is possible to state on the basis of existing data that xanthines exert their antiasthmatic effects via mechanisms unrelated to adenosine antagonism.

RENAL ACTIONS

Of the many pharmacological actions of methyl xanthines the diuretic effect was the first to be applied clinically

(40). The therapeutic value of this effect is today considered as limited but the pronounced acute diuretic effect is sometimes utilized in the treatment of acute heart failure.

Theophylline and caffeine induce a natriuretic effect that cannot be explained only as a result of increased cardiac function (41). Instead other mechanisms such as antagonism of adenosine (42,43) have been proposed to explain their diuretic effects. Adenosine has been suggested to mediate a feedback regulation of glomerular filtration rate that is activated by changes in distal tubular flow or sodium delivery (44). This is consistent with the observation that adenosine causes vasoconstriction in renal afferent arterioles (45). As a significant support for this hypothesis, enprofylline, over a wide range of dosis, was devoid of diuretic effects in rats (12).

A comparison of the acute effects of enprofylline, placebo and theophylline has been performed in papers IV and V. Each trial involved eight healthy volunteers and the different treatments were given intravenously over three different days applying randomized, double-blind, cross-over technique. The doses 1.5 mg/kg and 5.0 mg/kg of enprofylline and theophylline, respectively, resulted in plasma concentrations at the lower end of the therapeutic range and a ratio of roughly one to five. Hence the active drugs were given in equibronchodilating doses.

A further check on the potency ratio was provided by the simultaneous measurement in paper V of an independant smooth

muscle effect namely relaxation of the lower esophageal sphincter pressure (LESP). The similar degree of relaxation is in agreement with the results on the bronchi.

The diuresis was recorded by sampling urine in two hour portions (one portion in paper V and two in paper IV) after the infusion. The subjects voided before the infusion and had to abstain from drinking eight hours prior to the experiment. In paper IV the amount voided after two hours was substituted by asking the subjects to drink the same amount of water. The urine content of electrolytes was determined by flame photometry. In both papers a marked diuretic effect was recorded after theophylline while no effects could be detected after administration of enprofylline. Theophylline increased the amount of excreted sodium but had no effect on the amount of excreted potassium. Enprofylline had no effects on these variables.

The results in rats (12), the fact that the xanthines were given in equismooth muscle relaxant doses, and the equal effects of enprofylline and placebo strongly suggest that the difference between enprofylline and theophylline observed in this studies is qualitative. These data give further support to the hypothesis that adenosine may be involved in the regulation of the kidney function under normal physiological conditions.

METABOLIC EFFECTS

The most consistent finding concerning the metabolic effects in man of theophylline and caffeine is the release of free-

fatty acids (FFA) (46,47,48,49,50,51,52). This effect and elevation of glucose and renin and possibly glucagon and insulin have been proposed to be secondary to another metabolic action by xanthines namely catecholamine release from the adrenal medulla (52).

In man, however, data on catecholamine release are equivocal. Robertsson et al (53) found an increase of the plasma levels of adrenaline and noradrenaline but not of dopamine after 250 mg of caffeine given orally. Patwardan et al (46) found an increased urinary excretion of adrenaline and dopamine but not noradrenaline after the same dose of caffeine. Highbee et al (54) reported plasma dopamine levels to be unaffected but found an increase in plasma adrenaline and noradrenaline after oral administration of theophylline (5.0 mg/kg). Vestal et al (52) found a slowly developing increase of both adrenaline and noradrenaline plasma concentrations at theophylline levels above 10 $\mu\text{g/ml}$. The increase did not, however, coincide with maximum in theophylline concentration. The authors also admit (privat communication) that the subjects were not allowed to void during the experiment. This could in principle have an effect on the plasma levels of the catecholamines. On the other hand, Jung et al (50), reported no significant changes in plasma adrenaline or noradrenaline after i.v. or oral administration of caffeine, nor did Elkins et al (55) find any effects of oral caffeine on urinary excretion of adrenaline or noradrenaline.

The study in paper IV was undertaken to investigate the metabolic effects of i.v. enprofylline, theophylline and

placebo in eight healthy volunteers. The trial was performed over three different days with double-blind, randomized, cross-over technique. The drugs were given during 20 minutes and the doses were the same as in paper V that has been shown to result in equi-smooth muscle (bronchi and esophagus) relaxant concentrations. Plasma catecholamines were analysed with a specific HPLC method with amperometric detection (56,57). The limit of detection is 40, 80 and 120 fmol, respectively, for noradrenaline, adrenaline and dopamine, and the interassay coefficient of variation c:a 15% at plasma catecholamine levels around 1 nmol/L. Measurement of nonesterified (free) fatty acids (FFA) was performed with use of a commercially available kit (NEFA-C-test, Wako, Osaka, Japan). After an enzymatic reaction, the amount of FFA was quantified by colorimetry. Plasma glucose was measured with a glucose oxidase method (58) and plasma insulin, glucagon and renin were determined radioimmunologically; insulin with a Phadebas Insulin Kit (Pharmacia, Sweden), glucagon according to von Schenk and Nilsson (59) using antiserum E7, and renin according to Karlberg and Tolagen (60). Theophylline, but not enprofylline, markedly increased the plasma levels of FFA while neither theophylline nor enprofylline had any effects on the other metabolic variables. Hence the present study shows that theophylline and enprofylline at plasma levels that produce bronchodilatation may not be associated with changes in plasma catecholamines. These findings do not contradict the possibility that theophylline-induced elevations of glucose and renin (and possibly glucagon and insulin) in plasma, when they occur are secondary

to catecholamine release. On the other hand, contrary to what has been suggested by others (52,1), these data indicate that lipolysis after theophylline is not necessarily produced via raised plasma levels of catecholamines.

The fact that a non-blocking xanthine like enprofylline does not produce release of FFA suggests that adenosine receptor antagonism by theophylline may be involved in this action. As a matter of fact this mechanism has earlier been proposed based on the observation that adenosine in vitro has antilipolytic effects that are antagonised by theophylline already at concentrations of 2 $\mu\text{g/ml}$ (61,62).

GASTROINTESTINAL EFFECTS

Theophylline and caffeine influence both motility and secretory processes throughout the gastrointestinal tract. However, the two most carefully studied effects are relaxation of the lower esophageal sphincter pressure (LESP), (63,64) and effects on gastric secretion (65,66,67).

The effects on LES are pronounced at bronchodilating plasma concentrations in agreement with the clinical observation that treatment with theophylline of obstructive lung disease may result in gastric reflux (68,69).

The effects on LESP of intravenous theophylline, enprofylline and placebo were compared over three different days in paper V with randomized, double-blind, cross-over technique in eight healthy volunteers. The doses 1.5 mg/kg of enprofylline and 5.0 mg/kg of theophylline resulted in roughly equibron-

chodilating plasma levels i.e. in a ratio of one to five. LESP was measured before and after the infusion by three Beckman Pressure Transducers attached to a water perfused tube. Pressure was monitored with 1 cm intervals using the stationary pull through technique (70). End expiratory values obtained at the respiratory inversion point were considered to represent LESP and the mean of the three pressures was calculated. As gastrin is known to have an influence on LESP its plasma concentration was simultaneously measured.

A similar degree of relaxation of LESP was obtained after enprofylline and theophylline both in absolute figures and in percentage relative to the starting value. The fact that both an adenosine blocking and an adenosine non-blocking alkyl-xanthine are effective relaxants suggests that adenosine receptor antagonism is not involved. As no effects were seen on the plasma concentrations of gastrin of either xanthine the effects are unlikely mediated via this hormone. Instead the similar degree of relaxation after enprofylline and theophylline at equi-bronchodilating concentrations suggests that relaxation of LESP is mediated via the same cellular mechanism as relaxation of airway smooth muscle.

The ability of theophylline to stimulate gastric secretion has lead to the recommendation that it should be used with caution in patients with peptic ulcer. No really satisfactory theory of the mechanism behind the secretory effect exists. This is in part due to the large species-variation which has made it difficult to estimate the relevance of data obtained in animals.

The possibility that theophylline and enprofylline may have separate effects in man was addressed in paper V. The same design as in the investigation of effects on LESP was applied with identical doses. Gastric secretion was collected via a nasogastric tube during eight 15-minute-intervals and the xanthines were infused during collection of the fifth portion. Theophylline, but not enprofylline, significantly increased volume of gastric secretion with an increase in H^+ -concentration and increases in the amounts of excreted sodium and potassium. The effect of theophylline apparently was not due to gastrin release because the plasma levels of gastrin were unchanged.

The results actually suggest that an adenosine mechanism may be involved and hence that adenosine may play a role in the regulation of gastric secretion in man under normal physiological conditions. As a matter of fact data have since been presented (71) showing that intrarterially administered adenosine may abolish histamine and methacholine induced stimulation of gastric secretion in dogs and that simultaneous administration of theophylline eliminates the effects of adenosine.

Further investigations of the role of adenosine in the regulation of gastric secretion seem motivated on the basis of this information.

PHARMACOKINETICS

Enprofylline is metabolized to a low degree in rats (11) and man (16). It is excreted as unchanged drug via the

kidneys and has a short plasma half-life (around 2 hours in young, healthy, volunteers; 16). This pharmacokinetic profile is different from those of theophylline and caffeine that are metabolized to a high degree by the liver.

An outstanding problem in the therapeutic applications of theophylline and caffeine has been the intra- and interindividual variations of their liver metabolism (2,18,19). One may then wonder whether these variations are less in the case of enprofylline. Several questions can be raised in this context;

- 1) what is the half-life of enprofylline in a normal population of patients?
- 2) is it possible to control the plasma levels of enprofylline by varying infusion rates?
- 3) how is enprofylline absorbed from the gastrointestinal tract?

Question number 1 is impossible to answer on the basis of the data collected in a few patients. However, in paper I an estimate of the half-life in nine asthmatic patients was obtained by monitoring plasma levels during 12 hours after intake of plain tablets of enprofylline. A mean plasma half-life of 113 minutes was found in good agreement with results obtained in young, healthy volunteers (16). As remaining absorption cannot be excluded during the " β -phase" the obtained value only constitutes an upper bound.

An estimate of clearance and volume of distribution was obtained in six asthmatic patients in paper II. Enprofylline (1.5 mg/kg) and theophylline (5.0 mg/kg) were infused during 20 minutes and plasma levels were monitored during six

hours. The obtained values on clearance and volume of distribution agree well with results obtained in healthy volunteers (16). The calculations (72) are based on the assumption of linear kinetics i.e. that the obtained plasma levels $C(t)$ are linearly related to the given dose $d(t)$ and that the system is time invariant. Under these assumptions $C(t)$ can be written as a linear functional of $d(t)$ i.e.

$$C(t) = \int_0^t F(t-t^1) d(t^1) dt^1 \quad \text{where } F \text{ is an unknown function.}$$

For a bolus injection $d(t)$ is proportional to the Dirac function $\delta(t)$ defined by $\delta(t) = 0$ for all $t \neq 0$ and

$$\int_0^\infty f(t^1) \delta(t^1) dt^1 = f(0) \quad \text{for any function } f.$$

Hence for a bolus injection

$$C(t) = \int_0^t F(t-t^1) \delta(t^1) dt^1 = F(t) \quad \text{that is } F \text{ can be}$$

determined by giving a short infusion.

Knowing F one is able to calculate the resulting plasma concentrations from any infusion regimen. This possibility was utilized in paper II to simulate plasma concentration curves for a loading infusion followed by a maintenance infusion.

The simulated plasma concentration curves indicate similar distribution kinetics for enprofylline and theophylline. There was also good agreement between the measured and simulated plasma concentration curves suggesting that if

infusion rates are corrected for the short half-life of enprofylline the plasma levels can be controlled just as for theophylline.

The absorption of enprofylline from the gastrointestinal tract was investigated in paper VI. Three different doses of enprofylline as solution were given orally to six healthy volunteers and plasma levels were monitored during 10 hours after drug administration. The total amount of drug absorbed from the gastrointestinal tract was estimated by measuring urine recovery of unmetabolized enprofylline.

The results revealed that enprofylline is well absorbed. However, the higher dose levels resulted in the occurrence of double or multiple peaks in plasma levels.

In a second part of the study six healthy volunteers received the same dose of enprofylline at three different levels in the gastrointestinal tract. A solution of enprofylline was given orally, via a duodenal catheter, or rectally through a Foley catheter. The obtained data showed a fast and complete absorption from duodenum and a slow but reproducible absorption from colon, while large variations in time to peak in plasma concentration were seen after the oral administration.

Hence enprofylline appears to be poorly absorbed from the stomach while a good and reproducible absorption takes place from the intestines. This observation explains the multiple peaks in the first part of paper VI and it also provides an explanation of the distributions of time to peak in plasma concentration seen in paper I.

GENERAL DISCUSSION

Pharmacodynamics

Theophylline and caffeine antagonise cell surface receptors of adenosine in a series of tissues (42,7,8,9,14). As the role of adenosine under basal physiological conditions as well as ischemia and sympathetic over stimulation is partly unknown (73,74) it is still uncertain to what extent the action of methyl xanthines can be explained on the basis of this mechanism. One way to investigate the clinical relevance of these ideas is to compare the pharmacodynamic properties of theophylline and enprofylline (12), a xanthine with a negligible ability to antagonise adenosine (13,14,15). This possibility has previously been utilized to discuss bronchodilatation, slight CNS-stimulation (36,37,38) and some cardiovascular properties (75) of xanthines in man.

In the present thesis this program has been continued to obtain further information concerning the bronchodilating properties and to investigate other antiasthmatic aspects of xanthines i.e. their effects on immediate and late allergen induced bronchoconstriction. The same idea has been applied to gain insight into the diuretic and metabolic effects of xanthines. Two gastrointestinal effects, namely relaxation of lower esophageal sphincter pressure and stimulation of gastric secretion have also been investigated.

Two types of actions of xanthines have been seen. The first group of effects (bronchodilatation, vasodilatation,

effects on allergen induced bronchoconstriction and relaxation of the lower esophageal sphincter) contains actions where theophylline and enprofylline have qualitatively similar effects. The only difference is that enprofylline appears to be roughly five times more potent. The other group contains effects (CNS-stimulation, diuresis, FFA-releasing effects, and effects on gastric secretion) where the two xanthines seem to have qualitatively different effects.

The cellular mechanism behind the first group of effects is unknown, while the second group contains effects for which adenosine receptor antagonism can be proposed as an explanation. As a corollary, adenosine appears to be involved in the regulation under normal physiological conditions of the effects in group two.

Pharmacokinetics

The basic pharmacokinetic parameters of some xanthines including enprofylline are given in the table below based on a compilation of Zuidema et al (76) and data obtained in this thesis.

	$T_{\frac{1}{2}}$ (h)	V_d (l/kg)	Degree of metabolism	Oral bio-availability
Theophylline	7	0.4	90%	~100%
Caffeine	4	0.5	90%	~100%
Proxiphylline	7	0.5	75%	~100%
Diprophylline	2	0.6	~0%	~70%
Acephylline	1	0.5	~0%	~0%
Enprofylline	2	0.5	~0%	~100%

While the volume of distribution is the same for all the xanthines two groups can be distinguished regarding half-life and degree of metabolism. One group consists of xanthines with a relatively long half-life and a high degree of metabolism while the other group includes xanthines with a short half-life and no metabolism. It would be interesting to know how the relative variations in half-life of the two groups compare. Theoretically, one could speculate that the group with no metabolism should show less variations. However, this is far from obvious. After all it may very well be that the problem of variations in liver function has been translated to a question concerning variations of renal function. As these variations are poorly understood data on this issue would be interesting.

The absorption of enprofylline from the small intestines and colon appears to be good while the absorption from the stomach is negligible. Is this a common feature of xanthines with a good oral bioavailability or is the general pattern more complicated? Information on this problem could be valuable to discuss various galenic possibilities in the clinical applications of xanthines.

The future clinical value of enprofylline

The pronounced antiasthmatic properties of enprofylline in combination with its lack of massive CNS-stimulation (77), lack of diuretic effects, and short half-life seem to be

suitable for i.v. treatment of acute asthma. On the other hand, the future clinical value of enprofylline in oral maintenance therapy of obstructive lung disease will depend on the possibility to develop an adequate SR-formulation. The good absorption from the intestines seems to be favourable for such a galenic work.

During oral maintenance therapy the lack of slight CNS-stimulation and gastric secretory effects could be of clinically significant value. On the other hand, one needs more information concerning the frequency of adverse reactions such as headache and nausea to be able to more accurately estimate the future clinical value of enprofylline.

The lack of metabolism of enprofylline could mean less variability of clearance and thereby a simpler administration as compared to theophylline. This possibility has to be investigated in a large group of patients before any clinical conclusions can be drawn. However, data obtained in uremic patients (75), a potential risk group when treated with enprofylline, indicate that measurement of creatinine clearance could serve as a tool to determine the clearance of enprofylline in this group of patients.

Hence, a xanthine with the pharmacokinetic profile of enprofylline carries the potential to improve the present xanthine therapy, but many questions concerning variations, interactions, and groups of patients with an extreme excretion of enprofylline, have to be settled to fully understand the clinical implications of such a pharmacokinetic profile.

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I

Enprofylline – Effects of a New Bronchodilating Xanthine Derivative in Asthmatic Patients

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The bronchodilating effect of two doses of peroral enprofylline was compared with placebo in 24 asthmatic patients. Enprofylline produced significantly greater bronchodilatation than placebo. A dose of 2 mg/kg b.wt. and 4 mg/kg b.wt. caused a mean maximal increase in FEV₁ of 26% and 35%, respectively. The degree and the incidence of headache and nausea were estimated by means of a scoring system. Dose-related effects on both parameters were observed. Other side effects were negligible. In seven patients the mean plasma half-life of enprofylline was found to be 113 min. It is suggested that enprofylline should be studied further in patients suffering from obstructive lung disease.

Key words: adenosine antagonism; bronchodilator; enprofylline; half-life.

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Enprofylline (3-propyl xanthine) is a new compound which has proved to be a potent bronchodilator in man and animals (3, 6). In animals, enprofylline is devoid of theophylline-like CNS-stimulant behavioural effects (5, 6) and diuretic action (5). As it also has a negligible degree of metabolism (2), it should be able to lead to improvements in the asthma therapy with xanthines.

The present double-blind cross-over placebo comparison was designed to examine both the bronchodilator effect and the occurrence of significant subjective side effects of enprofylline in asthmatic patients. Plasma levels of enprofylline were analysed in all patients, and in a subgroup of seven, the plasma half-life was calculated.

PATIENTS AND METHODS

Thirty patients suffering from chronic, stable, obstructive lung disease gave their written informed consent to participate on 3 different days in a double-blind, cross-over comparison of placebo versus two doses of enprofylline.

The lung-function stability was controlled by demanding less than a 15% variation from the mean value of the morning recordings of FEV₁ obtained on the 3 examination days. If, after completing 3 examination days, the patient did not fulfil this stability criterion, he was asked to attend the clinic on a 4th day, when the lung function was again measured. The same criterion was then applied, taking the 4th day and 2 of the examination days into account. If the stability criterion was fulfilled, the odd day was repeated. The patient's lung function was otherwise regarded as unstable, and the patient was excluded from the study.

Twenty-four patients fulfilled the stability criterion as defined above; six of these 24 patients obtained a stable lung function after completing the 4th day of the trial (Fig. 1). Five patients had an unstable lung function and one patient left the trial for personal reasons.

The results are based on the 24 stable patients (14 males, 10 females). Their mean age was 46 years (range 24–55 years) and their mean weight 71 kg (range 54–111 kg). The mean duration of asthma was 16 years (range

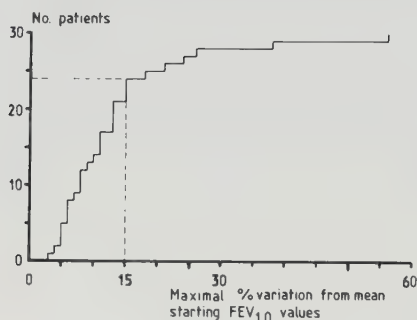


Fig. 1. Number of patients as function of their maximal daily variation. The stippled line corresponds to a maximal variation of 15%.

2–43 years). Sixteen patients suffered from extrinsic asthma defined as a typical anamnesis, a positive skin prick test to common allergens and specific plasma IgE concentration higher than 350 IU. Eight patients had intrinsic asthma.

The mean basal level of FEV₁ was 2.05 l, with a range of 1.25–3.8 l. Two patients had a basal level below 1.5 l. The mean expected level (FEV₁) was 3.41 l, with a range of 2.49–4.55 l. Basal FEV₁ in percent of the expected value was 60.1%. The mean reversibility (increase in FEV₁ 5 min after the inhalation of two puffs of terbutaline aerosol) was 32% (range 20–69%). All patients used sympathomimetics, 17 inhaled beclomethasone, 13 patients received theophyllines, four patients sodium cromoglycate, and two patients oral prednisone.

No patients suffered from heart, liver or kidney diseases, nor did they have any current infections or a history of migraine or seizures. No pregnant women were included.

The patients were randomly given enprofylline in a dose of 2 mg/kg b.wt. or 4 mg/kg b.wt. or placebo on 3 different days. The tablets looked identical in order to maintain the double-blind character of the study.

The two different doses and the placebo were coded for each patient and dispensed in a ran-

domized sequence for the 3 days. A duplicate set of tablets was prepared in case the patient needed to repeat 1 day because of instability. The code was known only to a pharmacist who was not involved in patient care or in the evaluation.

All oral medication, except glucocorticoids and beta-receptor stimulants, had to be discontinued 56 h before attendance at the clinic. Ten hours before the visit, the patients were taken off oral beta-receptor stimulants and spray treatment with sympathomimetics, anti-muscarinics and/or glucocorticoids. If necessary, a rimiterol spray (Pulmadil®) was permitted up to 4 h before the visit to the hospital. On each visit the patient was asked about his drug intake during the last 24 h before attending the clinic.

Beverages containing xanthine-like compounds, i.e., coffee, tea, coca-cola and chocolate, were not ingested the preceding night nor on the day of the trial.

Data collection

The patients stayed in the clinic from 8.00 a.m., when the tablets were administered, until 12.00 noon. The maximal FEV₁ values of three attempts were read directly from graphs drawn by a Wedge Bellows spirometer (Vitalograph) immediately before drug intake and every half hour. All values were corrected to BTPS values. After each determination of pulmonary function, a 5 ml venous blood sample mixed with heparin (Venoject® system) was obtained from an antecubital vein. The plasma was separated by centrifugation (5 min, 3700 r.p.m.) and analysed for enprofylline content by HPLC technique. Blood pressure and heart rate were measured before and 2 and 4 h after drug intake.

Every 60 min the patients were questioned in a standardised manner about adverse reactions (headache, nausea and palpitations) experienced during the preceding hour. Symptoms were graded on the following scale: 0 = absent, 1 = slight, 2 = moderate, 3 = severe.

To determine the half-life of enprofylline in patients, blood samples were obtained from nine patients every second hour until 8.00 p.m.

Statistics

Differences were determined with the Student's *t*-test for paired and unpaired samples. A difference corresponding to a *P*-value of less than 0.05 was considered significant.

RESULTS

Spirometry

Fig. 2 shows the spirometric values obtained after the two doses of enprofylline and placebo. A dose of 2 mg/kg resulted in significantly ($P < 0.05$) higher mean values than those achieved with placebo after 60, 90, 120, 150 and 180 min. A dose of 4 mg/kg resulted in significantly ($P < 0.05$) higher mean values than those with placebo after 60, 90, 120, 150, 180, 210, and

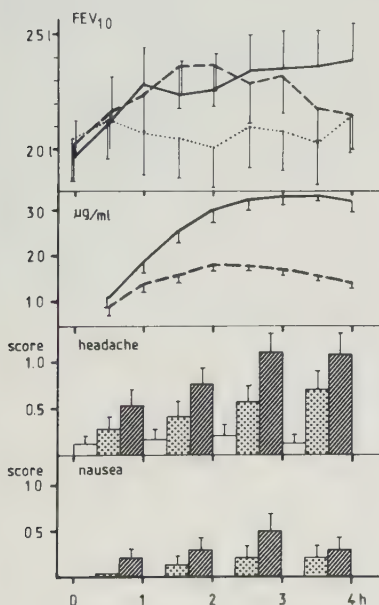


Fig. 2. Mean values for FEV₁, plasma concentrations, and side effects in 24 patients treated with enprofylline and placebo. Bars indicate SEM values.

Placebo: or □
 2 mg/kg: - - - - - or ▨
 4 mg/kg: ——— or ■

Table 1
 FEV₁ (litres) $\pm$ SD as function of time

Time (min)	Placebo	2 mg/kg	4 mg/kg
0	2.05 $\pm$ 0.92	2.02 $\pm$ 0.77	1.97 $\pm$ 0.77
30	2.13 $\pm$ 0.88	2.16 $\pm$ 0.78	2.12 $\pm$ 0.79
60	2.07 $\pm$ 0.78	2.24 $\pm$ 0.79	2.28 $\pm$ 0.78
90	2.05 $\pm$ 0.84	2.36 $\pm$ 0.88	2.24 $\pm$ 0.74
120	2.01 $\pm$ 0.81	2.37 $\pm$ 0.86	2.26 $\pm$ 0.76
150	2.10 $\pm$ 0.88	2.29 $\pm$ 0.86	2.34 $\pm$ 0.76
180	2.08 $\pm$ 0.82	2.32 $\pm$ 0.80	2.35 $\pm$ 0.77
210	2.03 $\pm$ 0.83	2.18 $\pm$ 0.73	2.36 $\pm$ 0.78
240	2.15 $\pm$ 0.78	2.15 $\pm$ 0.72	2.39 $\pm$ 0.79

240 min. A dose of 4 mg/kg also produced significantly ($P < 0.05$) higher mean values than 2 mg/kg after 210 and 240 min. (See Table 1).

The mean maximum increases $\pm$ SD of FEV₁ as a percentage of the initial values were 11.9 ± 10.8 , 26.0 ± 18.5 , and 35.2 ± 26.4 for placebo and enprofylline in a dose of 2 mg/kg and 4 mg/kg, respectively. The mean maximum increases in FEV₁ after 2 and 4 mg/kg of enprofylline were significantly ($P < 0.05$) higher compared with placebo.

Plasma levels and half-life of enprofylline

2 mg/kg of enprofylline resulted in a maximum plasma concentration of 1.83 ± 0.67 µg/ml (mean $\pm$ SEM) 120 min after intake, while 4 mg/kg resulted in a concentration of 3.32 ± 0.91 µg/ml 180 min after intake. The mean maximum plasma concentrations reached at any time after administration were 2.11 ± 0.61 µg/ml and 3.67 ± 1.16 µg/ml after 2 and 4 mg/kg respectively. The period of time required in order to reach plasma peaks showed a broad distribution around 2 h after 2 mg/kg and a biphasic distribution around 150 and 240 min after 4 mg/kg.

In two of the nine patients in whom blood samples were collected during a period of 12 h after intake, it was not possible to identify an elimination phase due to delayed absorption. The determination of the half-life ($t_{1/2}$) for enprofylline is therefore based on results from

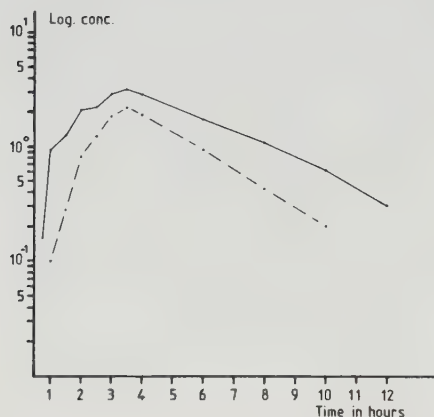


Fig. 3. Plasma concentrations obtained after two doses of enprofylline as function of time in a line-log scale.

2 mg/kg: -----

4 mg/kg: —————

seven patients (4 males and 3 females) with a mean age of 37 years (range 25–55 years) and a mean body weight of 75 kg (range 50–111 kg). A set of plasma concentration curves is illustrated in Fig. 3 for one patient. The value of $t_{1/2}$ was 113 ± 15 min (mean $\pm$ SEM).

Side effects

Determinations of blood pressure and heart rate showed no differences between placebo and active treatment with enprofylline.

Twelve patients complained of headache when given the low dose of enprofylline, 16 experienced headache after the high dose and three patients after the placebo. A dose of 2 mg/kg produced significantly ($P < 0.05$) higher scores than the placebo during the 4th h, whereas a dose of 4 mg/kg resulted in significantly ($P < 0.05$) higher scores than the placebo during the 1st, 2nd, 3rd and 4th h. Enprofylline in a dose of 4 mg/kg produced significantly ($P < 0.05$) higher scores than 2 mg/kg during the 3rd h (Fig. 2).

Five patients complained of nausea when given the low dose, nine with the high dose and no patients experienced nausea after receiving

placebo. One patient treated with the high dose vomited. 4 mg/kg resulted in a significantly ($P < 0.05$) higher score for nausea than the placebo during the 2nd, 3rd and 4th h (Fig. 2).

One patient developed palpitations after taking the placebo, while three developed palpitations after 4 mg/kg. One patient experienced slight dizziness after 2 mg/kg of enprofylline.

DISCUSSION

Various mechanisms have been proposed as explaining the pharmacological actions of theophylline. These include inhibition of the catabolism of cyclic AMP, mobilisation of intracellular calcium and antagonism of adenosine receptor-mediated effects (7). Of these mechanisms, only adenosine antagonism seems to be definitely produced at therapeutic concentrations of theophylline. Indeed, some well-known characteristics of theophylline, such as diuresis and CNS-stimulant behavioural effects, probably reflect adenosine receptor antagonism (1, 4). It is therefore particularly interesting that enprofylline did not, over a wide range of concentrations, antagonise the inhibitory effects of adenosine in smooth muscle (trachea) and nervous tissue (myenteric plexus) (5), and that it was devoid of theophylline-like natriuretic and CNS-stimulant behavioural effects (5). As enprofylline has, in addition, proved to be a potent bronchodilator in man and animals (6), it is suggested that adenosine antagonism is neither a necessary nor a desirable characteristic of xanthine anti-asthmatic drugs (5).

An earlier study, in which enprofylline was given as plain tablets on an empty stomach, showed a fast and complete absorption (2). But when enprofylline, as in this study, was given in the same type of tablets *after* breakfast, an irregular absorption pattern was obtained. This finding may be explained by the possibility that absorption of enprofylline from the stomach is negligible (2).

The spirometric data confirm that enprofylline is a bronchodilator. Due to the slow absorption of enprofylline, a clear dose response relation was not revealed during the 4 h of

observation. Significant adverse reactions were dose-related headache and nausea. It is known that non-users of theophylline may have transient adverse reactions when exposed to this drug. For this reason it is important to determine whether the number of theophylline non-users are overrepresented in the groups that complained of adverse reactions. Analysis revealed a small overrepresentation of patients who did not use theophylline in their daily medication in the group complaining of nausea, but no such finding in the group with headache. The cause of these side effects, their frequency and severity in relation to other bronchodilators, and the possibility that they are transient during long-term therapy cannot be settled on the basis of present information, but will be dealt with in future studies.

Enprofylline was shown to undergo a negligible metabolic degradation and has a high renal clearance (2). The pharmacokinetic information obtained in this study is consistent with previous results obtained from healthy volunteers (2).

Thus, the present study confirmed that enprofylline is a bronchodilator in asthmatic patients, that significant dose-related headache and nausea may occur and that the half-life of enprofylline in asthmatic patients is short.

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II

Intravenous Administration of Enprofylline to Asthmatic Patients

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Summary. In 6 asthmatic patients, the possibility of obtaining a steady state plasma level of 5 mg/l of enprofylline by administration of two constant rate infusions was examined. The simulated plasma concentration curves, based on information from preassessment of individual pharmacokinetic parameters, were in good agreement with the plasma levels obtained. The side-effects and bronchodilatation produced by enprofylline were compared to those obtained with theophylline at a steady state level of 15 mg/l. Enprofylline and theophylline caused a mean maximal increase in FEV_{1.0} of 14% and 2.6% per mg/l in plasma, respectively. Side-effects, headache, nausea and vomiting, became pronounced in 2 patients in whom the plasma enprofylline level was about 6 mg/l. No other serious adverse reaction was seen. It is suggested that enprofylline should be further evaluated as a possible anti-asthmatic drug.

Key words: enprofylline, theophylline; constant infusion, bronchodilator effect, adverse reactions, pharmacokinetics, asthmatic patients

Patients and Methods

Six patients (4 men and 2 women) suffering from reversible obstructive lung disease were informed about the trial and gave their written consent to participate in it. The mean age was 34 years (range 29–54 years) and the mean weight was 77 kg (range 58–95 kg). The mean duration of their asthma was 22 years (range 5–40 years). The mean basal FEV_{1.0} was 1.60 l (range 1.25–2.25 l); the expected mean normal FEV_{1.0} was 3.39 l (range 3.00–3.91 l). The mean reversibility of FEV_{1.0} was 39% (range 22–70%) measured 5 min after 2 puffs from a terbutaline aerosol.

Two patients had extrinsic asthma, defined as a typical anamnesis, positive skin prick test to common allergens and a significant specific plasma IgE concentration, and 4 patients suffered from intrinsic asthma. All patients used sympathomimetics, 2 patients took theophylline, and 5 patients used inhaled steroids.

The trial was performed as a double-blind, randomized crossover comparison of enprofylline and theophylline. Effects and side-effects were studied during constant infusion to produce plasma levels of 5 mg/l and 15 mg/l respectively. Certain basic, individual pharmacokinetic information was required in order to calculate the appropriate infusion rates. This was obtained on 2 separate days when enprofylline and theophylline were given intravenously for 20 min, in the doses of 1.5 and 5 mg/kg body weight, respectively, and blood samples were collected and analysed for drug content 0, 20, 40, 60, 120, and 360 min after the infusion. Wagner [9] has shown that the use of 2 infusions constitutes a safe and rapid way to obtain a steady-state plasma level; the initial infusion is given to reach the desired plasma level, and the second to maintain it.

Enprofylline is a potent bronchodilator when given orally to produce plasma levels between 2–5 mg/l [3, 4]. Dose-related adverse reactions, such as headache and nausea, may occur [3]. The drug is metabolized to a minor extent [2] and has high renal clearance, yielding a short half-life of around 2 h [2, 3].

The following study was performed to evaluate the suitability of enprofylline for intravenous use. The aims of the investigation were to examine the possibility of producing a plasma level of enprofylline of 5 mg/l by varying the infusion rate, and to compare the bronchodilator activity and side effects after i.v. enprofylline and theophylline.

For calculation of infusion rates without assuming any specific pharmacokinetic model, the following method was employed: the volume of distribution at steady-state (V_{dss}) and total body clearance (Cl) were calculated by a model-independent method [1]. By definition, at steady state the amount of drug eliminated per time unit is $C_{ss} \cdot Cl$, and the amount of drug in the body is $C_{ss} \cdot V_{dss}$, where C_{ss} is the plasma concentration. To maintain C_{ss} , the maintenance infusion rate must equal $C_{ss} \cdot Cl$. The loading dose is $C_{ss} \cdot V_{dss}$. Since the plasma concentration increases from zero to C_{ss} during the initial infusion, the average concentration during this period can be approximated by $C_{ss}/2$. To compensate for elimination during the loading infusion, the total infusion rate during the loading phase was calculated as $C_{ss} \cdot V_{dss}/T + (C_{ss}/2) \cdot Cl$, where T is the time of the loading infusion.

Deconvolution has been used to calculate the rate and extent of absorption of a drug [8]. The basic deconvolution equation can be used in the reverse direction to simulate the plasma concentration curves after various infusion schemes, using no assumptions other than dose-independent pharmacokinetics and knowledge of the plasma concentration profile after a short infusion. The duration T of the loading infusion was determined so as to avoid a high plasma peak immediately after the initial infusion.

The comparison of enprofylline and theophylline took place 14 days after assessment of the pharmacokinetic parameters. Day-to-day variation in lung function was controlled by requiring less than a 15% change from the starting value on the 2 test days. If, after completing the 2 days, the patient did not fulfil the stability criteria, he was asked to visit the clinic on a third day, and lung function was measured on arrival. The stability criterion was then applied, using the third day and 1 of the completed days. If the stability criterion was fulfilled, the odd-day treatment was repeated. All patients had stable lung function, although a third examination day was required for 3 patients.

The patients stayed in the clinic from 8.00 a.m., when the loading infusion was started. Pulmonary function was measured immediately before the loading infusion, at the beginning and 20 and 60 min after starting the maintenance infusion, and 5 min after two puffs of a terbutaline spray given after 60 min of maintenance infusion.

Measurements were made with a Wedge Bellows spirometer ('Vitalograph') running at 30 mm/s. After each pulmonary function test, venous blood 5 ml was collected in a heparinized tube (Venoject® system) from the contralateral antecubital vein. The plasma

was separated by centrifugation (5 min, 3700 rpm) and analysed for drug content by an HPLC technique [2]. At the end of the loading and maintenance infusions, the patients were questioned in a standardized manner about adverse reactions (headache, nausea and palpitations).

The data were analysed by Student's *t*-test, a difference corresponding to a *p*-value less than 0.05 being considered significant.

Medication

The ampoules were made to appear identical in order to maintain the double-blind nature of the study; they were provided by Draco Laboratories, Lund, Sweden. Two similar sets of ampoules were packed for each patient in case 1 examination had to be repeated.

All peroral medication, except for glucocorticoids and beta-receptor stimulants, were withdrawn 56 h before the visit to the clinic. 10 h before attending the clinic, patients stopped oral beta-receptor stimulants and spray treatment with sympathomimetics, anticholinergics or glucocorticoids. If necessary, a spray of rimiterol (Pulmadil®) was permitted up to 4 h prior to the visit to the clinic. At each visit the patient was asked about his drug intake during the previous 24 h. There was total abstinence from beverages containing xanthine compounds, i.e. coffee, tea, Coca-Cola and chocolate, over-night and on the test days.

Results

A. Pharmacokinetics

The values for total body clearance and V_{dss} obtained after a 20-min infusion are given in Table 1, and a typical set of plasma concentration curves is illustrated in Fig. 1. The simulated plasma concentration curves for various durations (T) of the loading infusion are displayed in Fig. 2. It can be seen that the loading infusion should be performed over at least 40 min to avoid a high peak. In order to achieve an adequate safety margin, T was fixed at 60 min. The simulated individual plasma concentrations and the values obtained during the investigations are given in Figs. 3 and 4.

B. Spirometry

Mean values of FEV_{1.0} as a function of time are given in absolute values in Fig. 5, and relative to the starting values in Fig. 6.

Subject 2

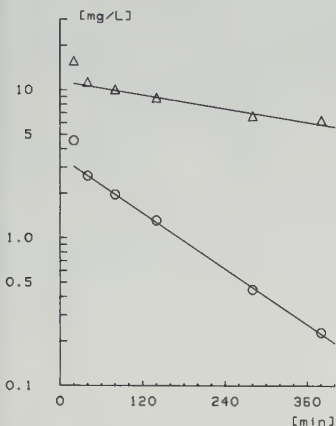
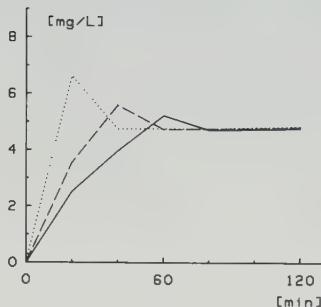


Fig. 1. Log plasma concentration versus time after a 20-min infusion in 1 patient. Theophylline: Δ ; Enprofylline: O ; Fitted line to the elimination phase: —

Enprofylline



Theophylline

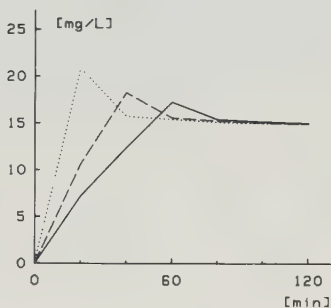


Fig. 2. Simulated concentration curves at different times after loading infusions of differing durations based on data from Fig. 1. 20 min loading infusion: 40 min loading infusion: - - - - - 60 min loading infusion: —

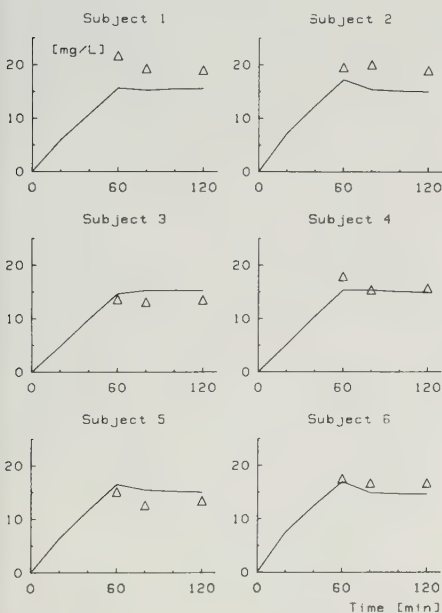


Fig. 3. Simulated (—) and measured (Δ) plasma concentration curves of theophylline

The mean $FEV_{1.0}$ at 60, 80 and 120 min, and after 2 puffs of aerosolized terbutaline, was significantly ($p < 0.05$) higher after each drug as compared to the starting value. There was no significant difference between the two drugs. The increase after two puffs of terbutaline was significant ($p < 0.05$) both after enprofylline and theophylline.

C. Adverse Reactions

After enprofylline administration, Patient 3 had a moderate headache during the second hour, followed by severe headache for 6 h. Patient 4 experienced severe nausea during the first h and a moderate headache during the second h; he vomited during the second h. Patient 5 had severe nausea during the 2 h of the test and experienced moderate epigastric pain.

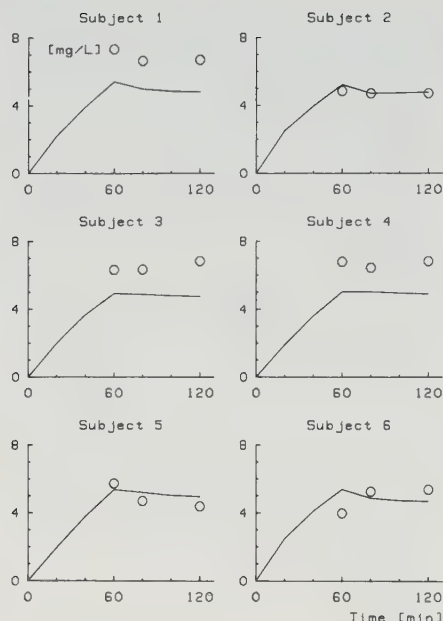


Fig. 4. Simulated (—) and measured (O) plasma concentration curves of enprofylline

When receiving theophylline, Patient 2 complained about headache, felt dizzy and vomited from 1 to 6 h after the start of the infusion. Patient 3 experienced 1–2 h of moderate tremor.

Discussion

In the treatment of acute asthma with theophylline, there is the problem of intravenous administration of a drug with a small therapeutic range. The problem is accentuated by the large individual variations in the metabolism of theophylline, and although careful infusion schemes have been worked out, serious side-effects, such as convulsions, may still occur [7]. Therapeutic benefit in the treatment of acute asthma should be obtained with a drug like enprofylline, which is a potent bronchodilator [3, 4], and is metabolized to a small extent [2]. As enprofylline, contrary to theophylline, does not antagonise the CNS-depressant effects of adenosine [6], it may lack some of the CNS-stimulant effects of theophylline [5]. Moreover, if other concentration-dependent xanthine-like

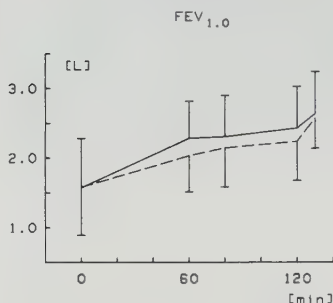


Fig. 5. FEV_{1.0} (mean $\pm$ SD) as function of time. Enprofylline: — Theophylline: - - -

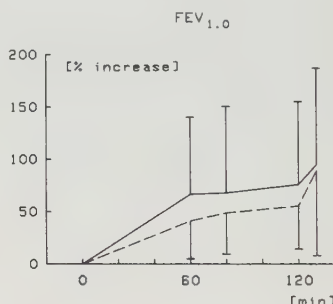


Fig. 6. Increase in FEV_{1.0} as a percentage of the starting value (mean $\pm$ SD). Enprofylline: — Theophylline: - - -

adverse reactions occur, a short half-life, like that of enprofylline, should be an advantage.

Before trying enprofylline in patients with acute asthma, information is needed about the changes in plasma levels when infusion parameters are varied, adverse reactions, and its bronchodilator effect after i.v. administration.

In the present study the possibility of achieving a steady-state plasma level of enprofylline of 5 mg/l by varying the infusion parameters was investigated. By aiming at the upper limit of the tentative therapeutic range of enprofylline, various dose-dependent pharmacokinetic effects were likely to be detected. Moreover, if there were no large deviations from the 5 mg/l level, investigation of adverse reactions at that level would be valuable.

Wagner's discussion has shown [9] that the use of 2 infusions constitutes a safe and rapid way to obtain a steady-state plasma level.

Table 1. Pharmacokinetic parameters of enprofylline and theophylline

Subject	Body weight [kg]	Total body clearance [l/h]		V_{dis} [l/kg]	
		Enprofylline	Theophylline	Enprofylline	Theophylline
1	95	17.2	6.22	0.62	0.49
2	85	15.4	3.89	0.38	0.44
3	58	24.4	5.10	0.54	0.42
4	79	22.1	3.67	0.55	0.44
5	74	9.8	3.96	0.52	0.41
6	71	17.2	6.78	0.47	0.51
Mean	77	17.7	4.94	0.51	0.45
$\pm$ SD	13	5.15	1.32	0.080	0.040

As the pharmacokinetic properties of enprofylline have so far been investigated in a limited number of patients [2, 3], the calculated infusion rates were based on a model-independent method. Benet [1] have shown how V_{dis} can be calculated just on the assumption of linear kinetics.

The simulated curves both for enprofylline and theophylline, with varying durations of the loading infusions, showed that safe infusions with minimal overshoot peaks could be obtained only when the infusions lasted for 40 min or longer. The calculated clearance of enprofylline (17.7 ± 5.15 l/h) agreed well with earlier results; the volume of distribution at steady state was somewhat smaller for theophylline than for enprofylline (0.45 VS 0.51 l/kg), and did not differ from previous findings concerning V_{dis} [2]. Thus, the present results suggest that, when differences in clearance and half-life are taken into account, the plasma levels of enprofylline can be controlled in a similar way to those of theophylline.

Earlier studies have indicated that enprofylline given orally to patients with moderate, reversible, obstructive lung disease is 3–5 times more potent than theophylline in its anti-asthmatic actions [3, 4].

When given i.v. to the same type of patient in the present study, enprofylline caused a 14% improvement per mg/l in plasma. The corresponding figure for theophylline was 2.6%. Hence, the results are in agreement with the suggestion, based on *in vitro* assay of bronchorelaxant potency in human airway preparations [6], that enprofylline is about 5 times more potent as a bronchodilator than theophylline.

Adverse reactions, headache and nausea, became pronounced in two patients who reached plasma levels of enprofylline exceeding 6.5 mg/l, and in 1 patient who reached 5.7 mg/l. No other alarming adverse reactions were seen.

In conclusion, therefore, the present data suggest that by varying the infusion rate, the plasma level of enprofylline can be controlled like that of theophylline. When enprofylline was given to patients with moderate asthma by a loading infusion for 1 h to produce a plasma level of 5 mg/l, substantial bronchodilatation was observed, although half the patients experienced headache or nausea. Questions remain about efficacy versus side-effects at lower steady-state plasma levels, or after shorter infusions, and they require study in future evaluation of the potential value of enprofylline as an anti-asthma drug.

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III

THE EFFECT OF THEOPHYLLINE AND ENPROFYLLINE ON ALLERGEN
INDUCED BRONCHOCONSTRICTION

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ABSTRACT

The effect on the allergen induced immediate and late bronchoconstriction, of theophylline and enprofylline (3-propylxanthine), a new xanthine derivative with negligible ability to antagonize adenosine, was studied in nine asthmatics. The patients were challenged three times, at weekly intervals, with the same dose of allergen, FEV₁ and sGaw were followed up to six hours after challenge. The drugs were given intravenously. Placebo was always given on the first occasion. Theophylline and enprofylline were administered on test day 2 and 3 with a double-blind, randomized, cross-over technique. One hour before the allergen challenge a loading dose was given during 60 minutes followed by a constant infusion during six hours. The loading infusion was 7.2 mg/kg b.w. of theophylline and 2.7 mg/kg b.w. of enprofylline. The maintenance dose was 74 mg/h and 71 mg/h, respectively. Theophylline and enprofylline both caused a minor initial bronchodilatation. Theophylline and enprofylline slightly but significantly inhibited the immediate bronchoconstrictory reaction after allergen inhalation. Theophylline

and enprofylline had a significant inhibitory effect on the late bronchial reaction. The mean plasma level of theophylline was 0, 10.8, 10.5 and 10.5 mg/l at 0, 1, 4 and 7 h after the start of the loading infusion respectively. The corresponding mean plasma levels of enprofylline were 0, 2.6, 2.7 and 2.7 mg/l. Theophylline and enprofylline caused headache in one patient. Two patients developed nausea and vomiting during the enprofylline infusion. The present data do not support the hypothesis that xanthines inhibit bronchoconstriction via adenosine receptor antagonism.

ABBREVIATIONS USED:

FEV ₁	: Forced expiratory volume in one second
Raw	: Airway resistance
TGV	: Thoracic gas volume
sGaw	: Specific airway conductance

INTRODUCTION

It has recently been established that theophylline (1,3-dimethyl xanthine) at clinically relevant concentrations exerts specific antagonism at cell surface receptor sites for adenosine (1,2). This nucleoside may potentiate antigen-induced mediator release from human lung mast cells (3). Given as inhalation adenosine has shown to produce bronchoconstriction in atopic and non-atopic asthmatic patients (4). Different works have also suggested that anti-allergic and bronchodilator actions of xanthines reflect adenosine antagonism (4,5,6).

Enprofylline (3-propylxanthine) is a newly synthesized derivative shown to be a potent bronchodilator in patients with reversible airway obstruction (7). In contrast to theophylline, this xanthine is a poor adenosine antagonist and seems to be without a number of theophylline's extrapulmonary effects (8,9). Diuresis, tremor, restlessness, seizures and metabolic effects exemplify actions of theophylline, that are not shared by enprofylline and that may reflect adenosine antagonism (10,11).

By comparing the effects of theophylline and enprofylline it may be elucidated whether or not adenosine is involved in a particular pharmacological action of xanthines. We have therefore investigated the effect of theophylline and enprofylline on the immediate and late bronchoconstrictory reaction after allergen challenge in human asthmatic patients.

TABLE I : Clinical data on the nine asthmatic patients that took part in the study.

NR.	AGE	SEX	HEIGHT CM	WEIGHT KG	BASAL FEV ₁ % PREDICTED	PROVOCING ALLERGEN
1	24	M	173	70	87	DERMAT. PTER.
2	21	M	180	80	105	GRASS POLLEN
3	24	M	180	81	105	GRASS POLLEN
4	28	F	166	58	95	DERMAT. PTER.
5	31	M	174	81	94	DERMAT. PTER.
6	23	M	182	75	100	DERMAT. PTER.
7	29	M	177	78	95	CAT DANDER
8	22	M	174	54	102	DERMAT. PTER.
9	26	M	165	60	90	GRASS POLLEN

METHODS

Subject selection

Nine asthmatic patients, 1 woman and 8 men, aged 21 to 29 years, were selected for the study (Table I). All patients had positive prick skin and RAST tests for one or more allergens. During the diagnostic work up, they all had bronchial challenge with the allergen, thought to be clinically relevant. The quantity of allergen causing an immediate bronchoconstriction with an FEV_1 decrease between 20 and 50% of the prechallenge value was determined. Their prechallenge FEV_1 value was at least 80% of the predicted value on all test days. The allergens used are given in table I, together with the FEV_1 value on the first test day.

Study design

The trial consisted of 3 different test days, separated by at least one week. No drugs were allowed during the week preceding the test day. At each occasion the patients were challenged with the same allergen dose as the one found to cause an immediate 20 to 50% decrease of the FEV_1 during the diagnostic challenge procedure. The test day started at 9 a.m., one hour before allergen challenge (time -60), when a catheter was placed into an arm vein and a continuous infusion was started. A loading dose of the drug was given over 60 minutes, followed by a constant infusion of the drug

(maintenance dose) during 6 hours (from time 0 to time 360). On test day 1 a placebo was always given single blind. On test day 2 and test day 3 theophylline and enprofylline were administered in a double-blind, randomized, cross-over way. The technicians performing the bronchial challenge and the lung function measurements were not aware of the type of treatment given on the two days. The starting FEV_1 value on each test day, at 9 a.m. and before the start of the intravenous infusion, had to be within 80% of the expected value. Informed consent was obtained from all patients. The study design was approved by the ethical committee of the Academic Hospital of Ghent.

Allergen challenge

The allergen challenge was performed by the nebulization of the quantity of allergen, known to induce an immediate fall of FEV_1 between 20 and 50% of the prechallenge value. The bronchial challenge was started at 10 a.m. (time 0), i.e. after the loading dose of the drug. The allergen solution was nebulized with a Wiesbadener Doppel Inhalator, using an airflow of eight liter per minute. The patient inhaled the nebulized solution by tidal breathing during two minutes, with the outlet of the nebulizer in the mouth and the nose closed by a clip. The same allergen solution and the same nebulizer were used for each individual patient throughout the whole study.

Lung function measurements

Forced expiratory volume in one second (FEV_1) was measured using a water sealed spirometer (Expirograph Godart). Each given value represents the highest of three consecutive manoeuvres. Specific airway conductance (sGaw) was calculated from airway resistance (Raw) and thoracic gas volume (TGV). Raw and TGV were measured using a constant volume body plethysmograph (Jaeger). Each given value represents the mean of six consecutive manoeuvres.

Lung function measurements were performed before the start of the intravenous infusion (time -60), one hour after the start of the intravenous infusion and immediately before the allergen challenge (time 0), and fifteen (time 15) and thirty minutes (time 30), one hour (time 60), two hours (time 120), three hours (time 180), four hours (time 240), five hours (time 300) and six hours (time 360) after allergen challenge.

Drug dose and dosage

The drugs were given with an infusion pump. Saline was used as placebo. Enprofylline was administered in a loading dose of 2.7 mg/kg body weight over the first hour, followed by a maintenance infusion of 71 mg/hour during the next six hours. Theophylline was infused in a loading dose of 7.2 mg/kg body weight over the first hour and 74 mg/hour during the following six hours. Blood samples for drug analysis were taken

immediately before, one, four and seven hours after starting the infusion. Theophylline serum levels were analysed using a commercially available radioimmunoassay. Enprofylline serum levels were measured using a high performance liquid chromatography method.

Assessment of adverse reactions

Immediately before the bronchial challenge, i.e. one hour after starting the drug administration, and every following hour, the patients were asked if they had experienced any discomfort which they connected with the drug under investigation.

Statistical analysis

Non parametric tests (the signed rank test) were used for statistical analysis. A difference with a p value less than 0.05 is regarded as significant.

RESULTS

The starting lung function values, FEV_1 and $sGaw$, did not differ significantly on the three different test days and were all greater than eighty percent of the predicted value. The drug administration schedules resulted in a steady state plasma level from time 0, i.e. at the moment of the bronchial challenge, to the end of the experiment (figure 1). The mean serum levels of theophylline were 10.8, 10.5 and 10.5 mg/l at time 0, 180 and 360, respectively. The mean enprofylline serum levels were 2.6, 2.7 and 2.7, respectively. The ratio between theophylline and enprofylline concentrations from time 0 to time 360 remained approximately 4.

The lung function values are expressed as absolute values and as values relative to the immediate prechallenge value (time 0). This dual presentation is necessary if we want to take into account the bronchodilating capacity of both theophylline and enprofylline. Indeed, although these patients had an FEV_1 and a $sGaw$ within normal limits, both theophylline and enprofylline caused a slight bronchodilation (figure 2 and table II). The mean percentual increase in FEV_1 , after the loading dose was 1.5 (± 8.0 as SD) on placebo day, 12.5 (± 7.5) on the theophylline day and 8.5 (± 7.5) on the enprofylline day. The mean percentual increase in $sGaw$ were 27 (± 34), 60 (± 45) and 100 (± 83) respectively. During placebo treatment, an immediate and late bronchoconstriction after bronchial challenge were observed (figure 2 and 3, table II).

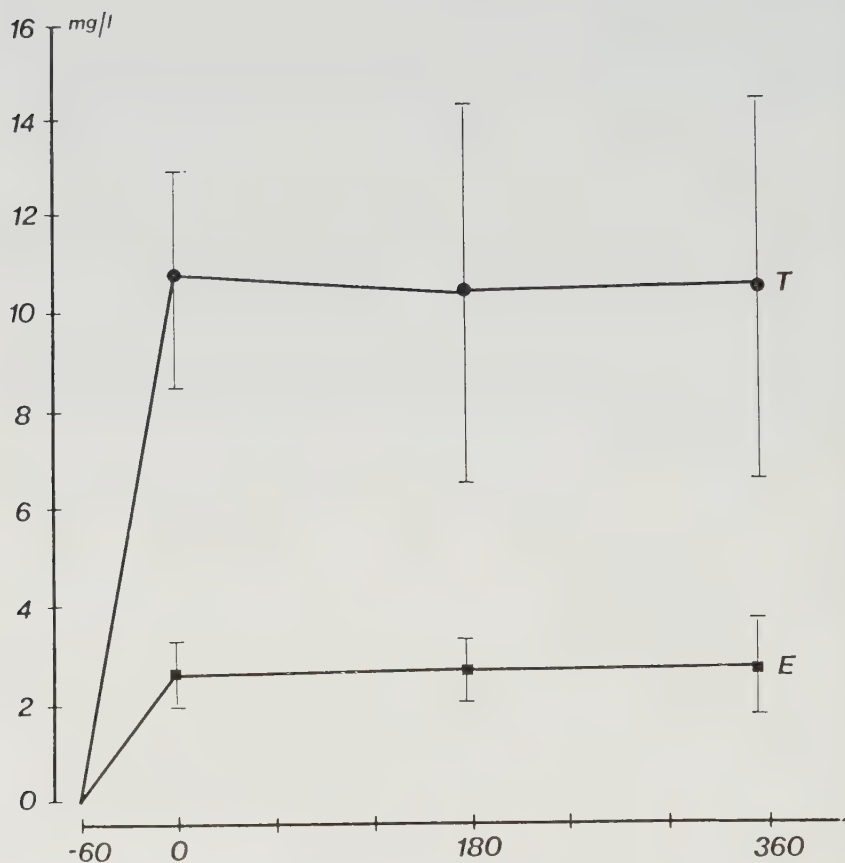


Fig. 1 Mean plasma levels (± 1 SD) of theophylline (T) and enprofylline (E) on the two test days.

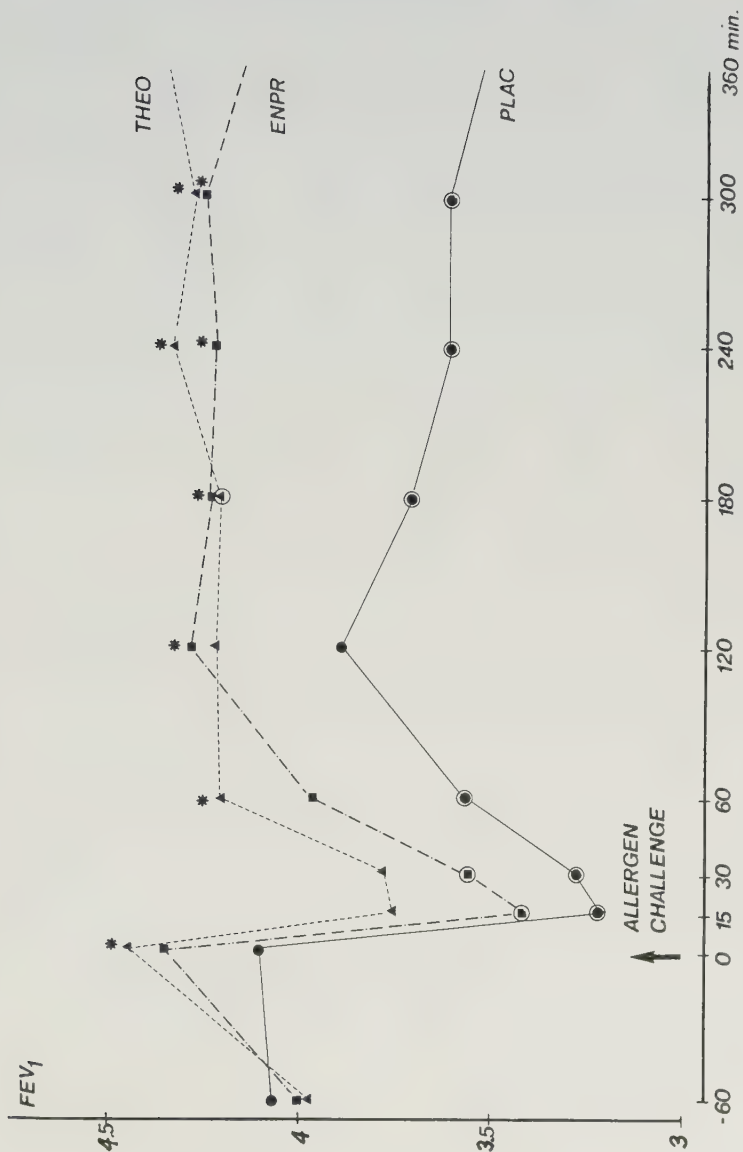


Fig. 2 Mean absolute values of the FEV₁ in nine asthmatic patients, challenged with allergen at time 0, and treated with placebo, theophylline or enprofylline from time -60 to time 360. * indicates a value significantly different from the placebo treatment. O indicates a value significantly different from the value at time 0.

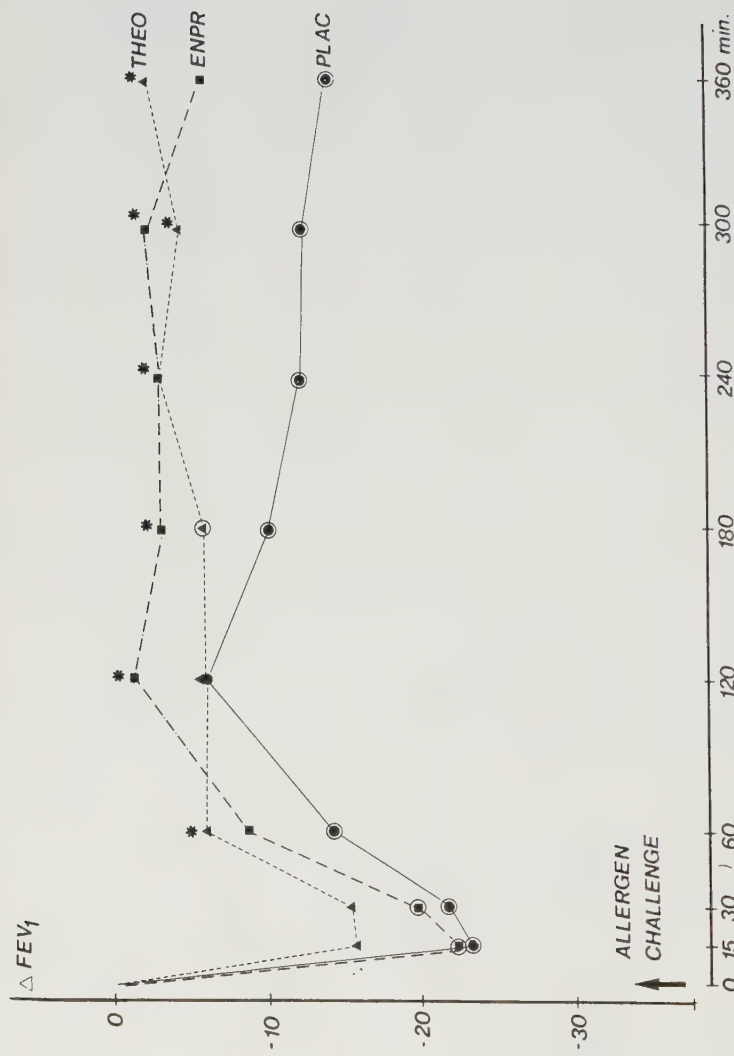


Fig. 3 Mean relative values of the FEV_1 (as percent of the FEV_1 value immediately before challenge) in nine asthmatic patients, challenged with allergen at time 0, and treated with placebo, theophylline or enprofylline from time -60 to time 360.

* indicates a value significantly different from the placebo treatment
 O indicates a value significantly different from the value at time 0.

Six out of the nine patients showed a decrease of the FEV_1 value beyond four hours after allergen challenge of more than fifteen percent of the prechallenge value. Neither theophylline nor enprofylline treatment had a significant inhibitory effect on the immediate decrease of the FEV_1 after allergen challenge. In contrast, both drugs inhibited significantly the immediate drop in sGaw (table II), even if we analysed the data relative to the value immediately before challenge. Both drugs also significantly inhibited the late bronchoconstrictory reaction measured by FEV_1 and sGaw (figure 2,3 and table II). Five of the six patients who manifested a late bronchial obstruction after placebo treatment, were significantly protected by theophylline and by enprofylline. The one patient showing a late bronchoconstriction during theophylline treatment was not the same patient as the one developing a late asthmatic reaction during enprofylline treatment. At no time was there a significant difference between the effect of theophylline and enprofylline on FEV_1 and sGaw changes.

With regard to side effects, headache was noted in one patient during enprofylline infusion and in another patient during theophylline infusion. Their maximal drug levels were 2.8 mg/l enprofylline and 11.1 mg/l theophylline, respectively. Two patients vomited during the enprofylline treatment. Their maximal enprofylline serum levels were 4.0 mg/l and 3.2 mg/l.

TABLE II : Mean absolute sGaw values in nine asthmatic patients, challenged with allergen at time 0, and treated with placebo, theophylline or enprofylline from time -60 to time 360.

* indicates a value significantly different from the value during the placebo treatment.

▲ indicates a value significantly different from the value at time 0.

TREATMENT	n	TIME	-60 min	0	15	30	60	120	180	240	300	360
PLACEBO	9	Mean (cm ² H ₂ O ⁻¹ sec ⁻¹)	0.111	0.157	0.053▲	0.059▲	0.088▲	0.121	0.106	0.107▲	0.095▲	0.093▲
		SD	0.052	0.053	0.040	0.047	0.054	0.064	0.060	0.064	0.067	0.070
THEOPHYLLINE	9	Mean	0.122	0.196*	0.137▲	0.154*	0.184*	0.187	0.174▲	0.163▲	0.164*	0.174*
		SD	0.042	0.043	0.056	0.073	0.074	0.063	0.041	0.058	0.050	0.058
ENPROFYLLINE	9	Mean	0.112	0.185	0.115▲	0.107▲	0.145▲	0.167	0.171*	0.175*	0.152	0.151
		SD	0.063	0.055	0.067	0.065	0.056	0.045	0.045	0.052	0.059	0.068

DISCUSSION

In this study of 9 asthmatic subjects both theophylline and enprofylline, at steady state plasma concentrations of about 10 and 2.5 mg/l, respectively, inhibited both the immediate and the late bronchoconstrictory reaction after allergen challenge. The protective activity was most pronounced during the late asthmatic reactions.

The effect of theophylline on allergen induced bronchoconstriction has not been extensively studied. Martin et al (12) observed that a treatment with oral theophylline, 125 mg every six hours, 36 hours before and during allergen challenge resulting in a mean plasma theophylline level before the challenge of 10 mg/l significantly reduced the immediate antigen-induced bronchospasm and mediator release. In a double-blind study Schultze-Werninghaus et al (13) demonstrated that 240 mg theophylline, given intravenously immediately before allergen challenge, significantly inhibited the immediate bronchoconstriction. The theophylline serum levels were not reported. Nissim et al (14) on the contrary, found no consistent effect of intravenous aminophylline pretreatment on the dose of antigen required to achieve a twenty percent fall in FEV_1 in five ragweed asthmatics despite therapeutic serum theophylline levels (12 mg/l). The same group of investigators (15) reported that treatment for at least 14 days with a slow release theophylline preparation, achieving therapeutic serum levels of 13.5 mg/l had only a marginal protective effect on allergen challenge, with regard to the

immediate bronchoconstriction. The results in the present study are in line with the observation that theophylline, at therapeutic plasma concentrations (10.5 mg/l), has only a slight protective effect on the immediate bronchoconstriction following allergen provocation in patients with bronchial asthma. This effect was significant using the sGaw as lung function parameter, not on the FEV_1 . The protective effect of theophylline was more pronounced on the late bronchial obstructive reaction following bronchial challenge.

Theophylline significantly inhibited the late bronchial reaction, both if we analysed the nine patients as a whole group or if we separated six patients developing a late bronchial reaction during placebo treatment. This observation may be related to the therapeutic efficacy of chronic theophylline treatment in asthma (16). The development of a late bronchial reaction after bronchial challenge with antigen is followed by a sometimes long lasting increase in non specific bronchial reactivity (17). The therapeutic prevention of the late bronchial reaction may abolish this increase in non specific bronchial reactivity. This, however, remains to be proven.

Enprofylline, at a steady state plasma concentration four times less than that of theophylline, has the same effect as theophylline on the allergen induced bronchoconstriction. The effective concentrations agree with the potency ratio between enprofylline and theophylline of about 5:1 observed for direct human bronchial smooth muscle relaxant effects and for bronchodilator effects in non-provoked asthma patients (7,8). In animals both enprofylline and theophylline have

been shown to inhibit bronchoconstriction induced by allergen and to inhibit increased microvascular permeability to macromolecules induced by mediators (18,19). Such anti-allergic and anti-inflammatory activities may have participated in the protective effect by the xanthines against development of the late asthmatic reaction after allergen inhalation.

The receptor antagonism of adenosine has been put forward as an explanation of the antiasthmatic effects of theophylline (4,5,6). Supporting this possibility, adenosine has been shown to potentiate the antigen induced mediator release from rat mast cells and human mast cells (2,5). Furthermore, theophylline at low and clinically relevant concentrations inhibits such potentiating effects of adenosine in vitro (1). These concentrations are considerably lower than the supratherapeutic molar concentrations which have been used to show direct inhibitory actions of theophylline on release of mediators from mast cells and basophils in vitro upon allergen challenge (20,21). Even at concentrations which are considerably larger than those obtained in the present study enprofylline has a negligible ability to antagonise adenosine actions. This has been shown in a variety of tissues harbouring different sub-types of adenosine receptors (smooth muscle, central and peripheral nervous tissues, cardiac tissues, fat cells, etc.) (9,19,22).

The inhibitory effects of xanthines on bronchoconstriction thus seem to be produced via mechanisms unrelated to adenosine antagonism. The role of adenosine as a lung autacoid remains

to be assessed. The presence of adenosine in lung tissue during allergen induced bronchoconstriction has not been documented. The bronchoconstriction after inhaled adenosine in asthmatic patients observed by Cushley et al. may not necessarily mirror the actions of endogenous adenosine (3). In isolated airway smooth muscle preparations adenosine produces both relaxant and slight constrictor effects (23). Besides its ability to potentiate mediator release this nucleoside has been shown at higher concentrations to inhibit release of mediators from inflammatory cells (2).

Headache, nausea and vomiting are recognized side effects of theophylline and are more frequently observed when the plasma theophylline levels exceed 20 mg/l (24). In the present acute study headache was observed in one patient out of the nine during each active treatment, but vomiting was only observed during enprofylline treatment. Studies in animals have shown that nausea may occur after administration of either enprofylline or theophylline, suggesting that this side effect may not reflect adenosine antagonism (11). The initial studies on tolerance in man have shown that enprofylline may produce headache and nausea (25). Further clinical studies are clearly needed before we know to what extent effects such as headache and nausea represent a drawback for the clinical application of enprofylline.

In conclusion, both the potent adenosine antagonist theophylline and the adenosine non-blocking xanthine derivative enprofylline protected asthmatic patients against immediate and late bronchoconstriction upon allergen challenge.

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IV

INCREASE OF PLASMA FREE FATTY ACIDS AND NATRIURESIS BY
XANTHINES MAY REFLECT ADENOSINE ANTAGONISM

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ABSTRACT

This study examines the hypothesis that adenosine is involved in diuretic and free fatty acid (FFA) releasing action of xanthines. The effects of theophylline (T), a potent adenosine antagonist, were compared with those of enprofylline (3-propyl xanthine, E), which has a negligible ability to antagonize adenosine. Eight healthy male volunteers were given E (1.5 mg/kg), T (5.0 mg/kg) and placebo (0.9 % saline, P) intravenously in a double-blind, randomized, cross-over investigation. Blood samples were analyzed for E, T, catecholamines (CA: adrenaline, noradrenaline and dopamine), FFA, renin, glucose, glucagon and insulin, and urine was collected at 2-h intervals. T (maximum plasma concentration $53 \pm 8 \mu\text{mol/L}$), but not E ($11 \pm 2 \mu\text{mol/L}$) caused an increase in FFA from 0.42 to 0.86 mmol/L after 90 min. Without affecting the urinary excretion of potassium T doubled natriuresis and the urinary volume compared to E and P. Neither T nor E had any effect on plasma CA or on any other of the metabolic parameters studied. E, but not T, produced small but statistically significant decreases in diastolic blood pressure (5 mm Hg) and increases in heart rate (3 beats/min). It is suggested that the difference between E and T concerning stimulation of FFA-release and natriuresis may be related to their different abilities to antagonize adenosine.

Key words: Theophylline; enprofylline; adenosine antagonism; free fatty acids; natriuresis; catecholamines.

INTRODUCTION

Recent investigations have shown that xanthine derivatives with antiasthmatic effects can be produced which differ from theophylline (and caffeine) in their profile of action (30). This is illustrated by the growing experiences in animals and man with enprofylline (PINN, 3-propyl xanthine). Like theophylline, enprofylline produces bronchodilation, vasodilation and relaxation of the lower esophageal sphincter, enprofylline being about 5 times more potent than theophylline (20, 24, 30, 33, 34). However, it has been shown that enprofylline, given in a wide range of doses to animals, is devoid of theophylline-like diuretic effects and CNS-stimulant behavioural actions (31, 32). The cellular basis for these and other differences in pharmacodynamic actions seems to be related to whether or not the xanthine is an adenosine receptor antagonist. Theophylline and caffeine at low and therapeutic concentrations are potent general inhibitors of adenosine actions (36). In contrast, enprofylline has a negligible ability to antagonize adenosine in a variety of cell types including smooth muscle, cardiac, nervous and adipose tissues (17, 30, 31, 32, 33, 34).

Inferentially, it has been suggested that differences in profiles of action between enprofylline and theophylline may reflect the involvement of adenosine (30). Hence, to test the hypothesis that adenosine is involved in diuretic and free fatty acid (FFA) releasing actions of xanthines in man we have studied effects of select single doses of theophylline and enprofylline on natriuresis and on plasma levels of FFA. Concomitantly, plasma catecholamines and some other metabolic variables were measured.

METHODS

Subjects and Procedures

Eight healthy male volunteers, mean age 34 years (range 25-48 years), mean weight 77 kg (range 70-88 kg) and mean height 181 cm (range 165-189 cm) participated in a double-blind, randomized cross-over investigation of the metabolic and diuretic effects of enprofylline, theophylline and placebo. The subjects gave their written consent to the study which was approved by the Ethical Committee of the University Hospital of Linköping. Enprofylline, theophylline and placebo were given on three different days and the subjects had to abstain from xanthine-containing food and beverages 72 h prior to each examination day, and were not allowed to eat or drink after 24.00 the preceding day.

Each examination day started at 08.00 in the morning, when the subjects after voiding and drinking 100 ml of water had two indwelling catheters inserted, one in each antecubital vein. After 30 min of rest, 1.5 mg/kg b.w. of enprofylline, 5.0 mg/kg b.w. of theophylline, or placebo (0.9% saline) were infused during 20 min. Blood samples were collected and analysed for drug content 20, 40, 60, 90, 120 and 240 min after starting the infusion. Blood samples for analysis of glucose, glucagon, insulin, catecholamines (adrenaline, noradrenaline and dopamine), renin and FFA were collected -30, 0, 20, 40, 60, 90, 120 and 240 min after starting the infusion. Heart rate and blood pressure (sphygmomanometer) were recorded concomitantly. Urine was collected in two portions (0-120 min, and 120-240 min) and analysed with respect to volume, and sodium and potassium content. The amounts of urine voided after 120 min were substituted by asking the subjects to drink the same amount of water.

Assays

Plasma enprofylline was determined by high performance liquid chromatography (HPLC). A detailed description of the analytical procedures is given elsewhere (Edholm and Heintz 1983, in preparation). The method is specific for enprofylline, and the inter-assay variation expressed as the coefficient of variation is less than 3.5 % at 0.4 and 3.0 mg/L. Theophylline in plasma was also determined by means of HPLC (11).

Plasma catecholamines were analysed with a specific HPLC method with amperometric detection (2, 3). The limit of detection is 40, 80 and 120 fmol, respectively, for noradrenaline, adrenaline and dopamine, and the interassay coefficient of variation ca 15 % at plasma catecholamine levels around 1 nmol/L.

Measurement of non-esterified (free) fatty acids (FFA) was performed with use of a commercially available kit (NEFA-C-test, Wako, Osaka, Japan). After an enzymatic reaction, the amount of FFA was quantified by colorimetry. Plasma glucose was measured with a glucose oxidase method (10) and plasma insulin, glucagon and renin were determined radioimmunologically; insulin Phadebas Insulin Kit (Pharmacia, Sweden), glucagon according to von Schenk and Nilsson (41) using antiserum E7, and renin according to Karlberg and Tolagen (22).

Data analysis

All data were analysed with respect to mean maximum and minimum values with and without subtraction of values at the start of the infusion. The statistical method was Student's t-test and a p-value less than 0.05 was considered as significant.

RESULTS

All subjects completed the study within three weeks. Variations in starting values for all parameters were insignificant.

Plasma concentration of drugs

Fig.1 shows the mean plasma concentrations of theophylline and enprofylline as a function of time. The mean plasma theophylline concentration 20 min after completion of the infusion was $52.7 \pm 7.70 \mu\text{mol/L}$. Corresponding value for enprofylline was $11.1 \pm 2.16 \mu\text{mol/L}$.

Free fatty acids

Fig.2 gives the plasma concentrations of FFA as a function of time. There was a rise in FFA concentration from a mean value of 0.42 mmol/L to a mean maximum value of 0.86 mmol/L obtained 90 min after infusion of theophylline. This change was statistically significant relative to both placebo ($p < 0.05$) and enprofylline ($p < 0.01$).

Diuretic actions

Fig.3 shows the urine production during the 2-h periods of sampling. The diuresis induced by theophylline was significant ($p < 0.001$ during the first 2 h and $p < 0.01$ during the next 2 h) relative to placebo and enprofylline. Fig.4 shows the amounts of Na^+ and K^+ in urine during each 2-h period. The amount of sodium

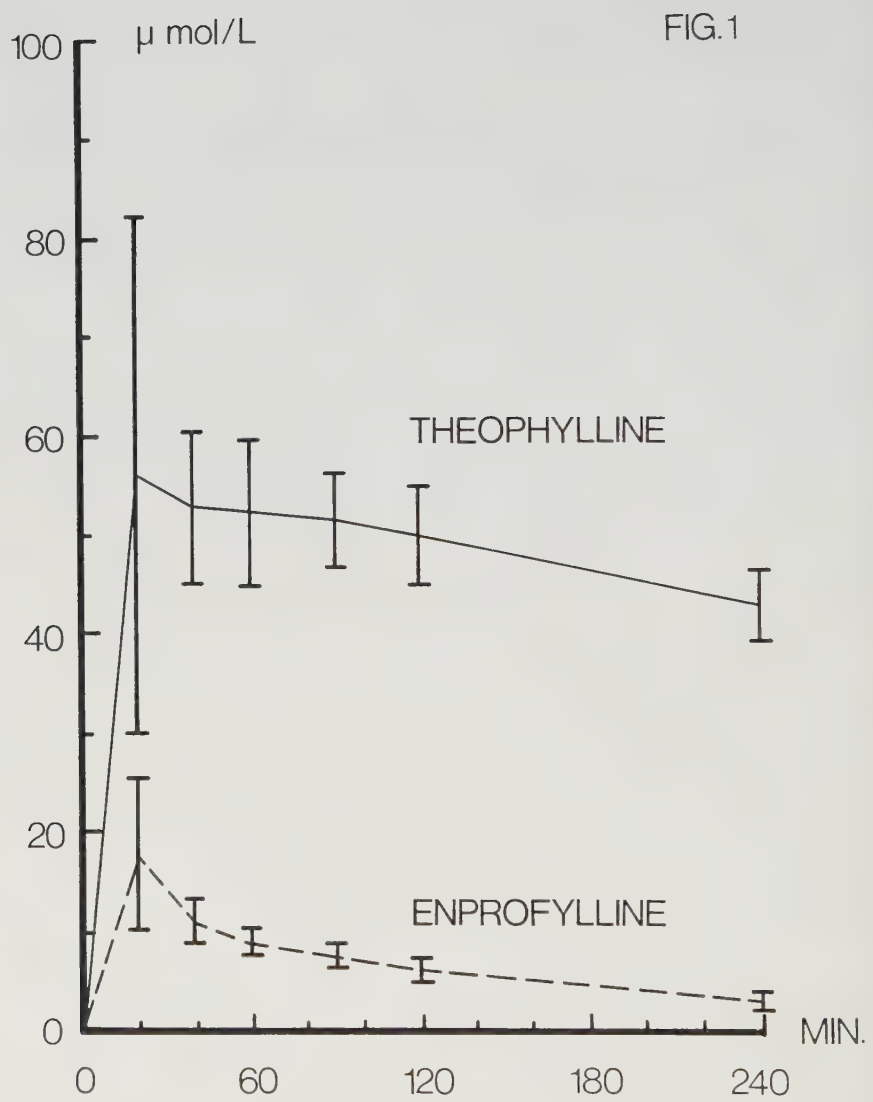


Fig. 1 Mean ($\pm$ SD) plasma concentrations of theophylline (—) and enprofylline (---) versus time after a 20-minute infusion starting at time 0.

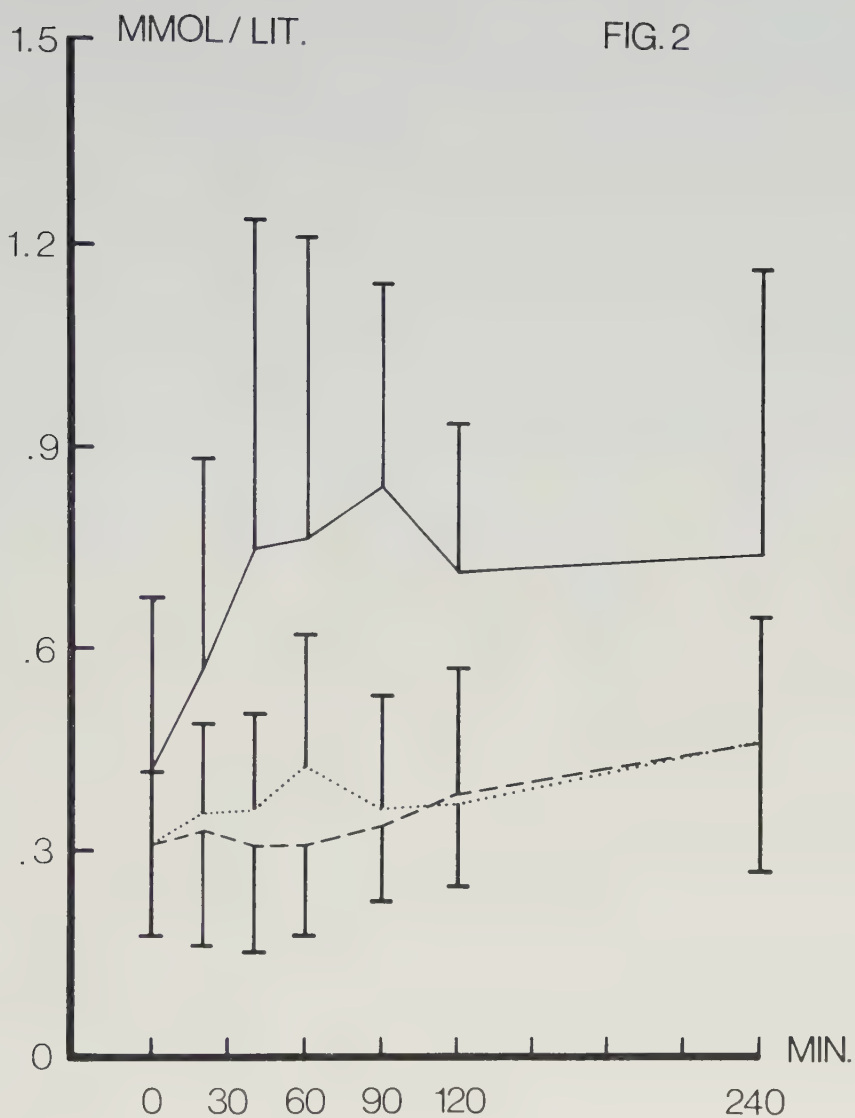


Fig. 2 Mean ($\pm$ SD) plasma concentrations of free fatty acids versus time after a 20-minute infusion starting at time 0. Theophylline —; enprofylline ----; placebo

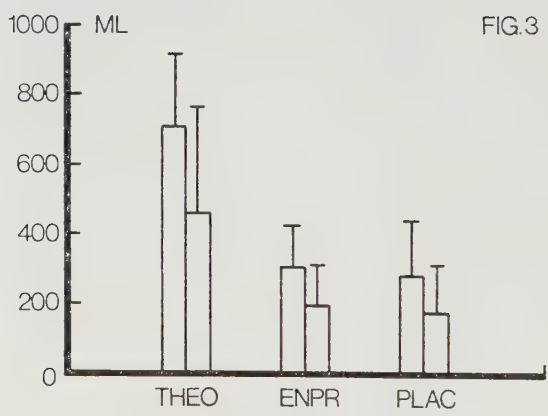


Fig. 3 Mean ($\pm$ SD) urine volume collected in two 2-hour portions after infusion of theophylline, enprofylline and placebo.

FIG.4

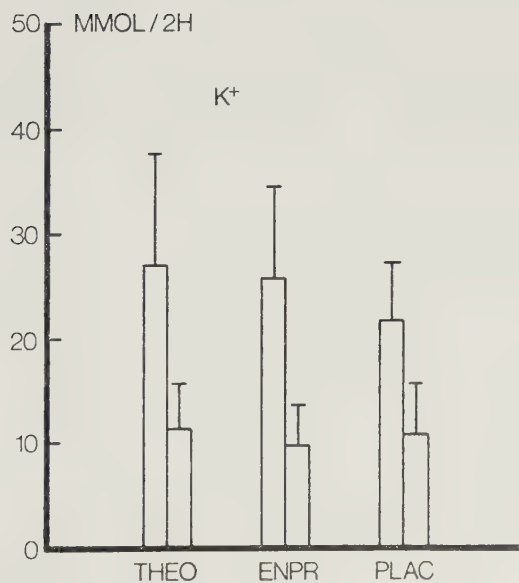
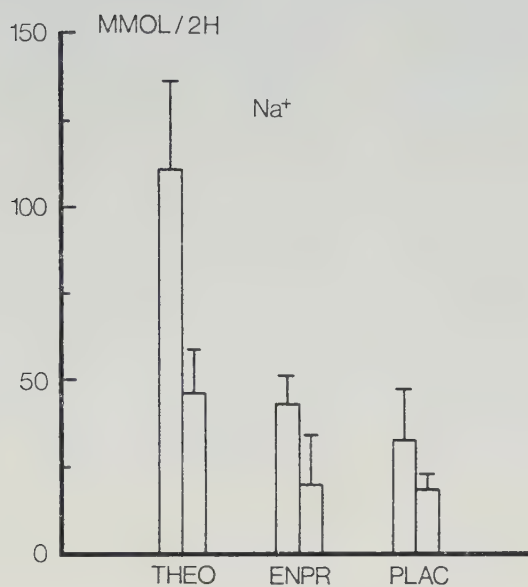


Fig. 4 Mean ($\pm$ SD) amounts of excreted Na^+ and K^+ in two 2-hour portions after infusions of theophylline, enprofylline and placebo.

excreted after infusion of theophylline was significantly ($p < 0.001$ during the first 2 h and $p < 0.01$ during the next 2 h) higher than after the placebo and enprofylline infusions.

Cardiovascular effects

A small (≈ 6 mm Hg), but relative to placebo significant ($p < 0.05$) drop in systolic blood pressure was seen after infusion of enprofylline. Relative to both placebo ($p < 0.01$) and theophylline ($p < 0.05$) there was a small, significant reduction in diastolic blood pressure (≈ 5 mm Hg). Infusion of enprofylline induced a small but relative to placebo significant ($p < 0.05$) increase in heart rate (3 beats/min). The effects of theophylline did not differ from those of placebo.

Plasma catecholamines

Table 1 gives the data on noradrenaline, adrenaline and dopamine. As can be seen there were no significant changes in the plasma levels of any of the amines.

Plasma renin, insulin, glucagon, glucose

Table 2 gives the data on renin, insulin, glucagon and glucose. No significant changes were found after infusion of theophylline, enprofylline or placebo.

Table 1. Plasma catecholamine concentration $\pm$ s.d. in nmol/L as function of time

Noradrenaline

	-30	0	20	40	60	90	120	240
Theophylline	1.34 ± 0.82	1.30 ± 0.83	1.86 ± 1.06	1.50 ± 0.87	1.46 ± 0.85	1.58 ± 0.53	1.70 ± 0.77	1.58 ± 0.70
Placebo	1.38 ± 0.51	1.08 ± 0.80	1.15 ± 0.67	1.73 ± 1.37	1.30 ± 0.92	1.43 ± 0.79	1.30 ± 1.04	1.70 ± 0.92
Enprofylline	1.20 ± 0.50	1.03 ± 0.49	1.50 ± 0.89	1.39 ± 0.73	1.51 ± 0.79	1.47 ± 0.55	1.44 ± 0.70	1.37 ± 0.65

Adrenaline

	-30	0	20	40	60	90	120	240
Theophylline	0.36 ± 0.24	0.26 ± 0.09	0.33 ± 0.19	0.32 ± 0.19	0.31 ± 0.18	0.31 ± 0.14	0.30 ± 0.11	0.25 ± 0.09
Placebo	0.34 ± 0.18	0.40 ± 0.35	0.34 ± 0.21	0.50 ± 0.58	0.24 ± 0.14	0.27 ± 0.13	0.36 ± 0.17	0.56 ± 0.72
Enprofylline	0.36 ± 0.16	0.43 ± 0.18	0.34 ± 0.11	0.93 ± 1.36	0.30 ± 0.18	0.31 ± 0.13	0.36 ± 0.21	0.31 ± 0.19

Dopamine

	-30	0	20	40	60	90	120	240
Theophylline	0.043 ± 0.040	0.030 ± 0.033	0.044 ± 0.042	0.088 ± 0.083	0.021 ± 0.036	0.064 ± 0.041	0.040 ± 0.039	0.045 ± 0.072
Placebo	0.084 ± 0.054	0.16 ± 0.27	0.11 ± 0.064	0.074 ± 0.039	0.070 ± 0.063	0.084 ± 0.064	0.061 ± 0.045	0.069 ± 0.070
Enprofylline	0.060 ± 0.045	0.039 ± 0.042	0.077 ± 0.071	0.068 ± 0.047	0.073 ± 0.040	0.11 ± 0.16	0.045 ± 0.040	0.037 ± 0.040

Table 2 Plasma concentration $\pm$ s.d. as function of time

Renin ($\mu\text{g/L}$)	-30	0	20	40	60	90	120	240
Theophylline	0.41 ± 0.35	0.32 ± 0.21	0.38 ± 0.20	0.36 ± 0.22	0.41 ± 0.27	0.45 ± 0.20	0.42 ± 0.20	0.48 ± 0.36
Placebo	0.35 ± 0.22	0.33 ± 0.26	0.24 ± 0.19	0.24 ± 0.16	0.20 ± 0.13	0.25 ± 0.14	0.26 ± 0.22	0.31 ± 0.24
Enprofylline	0.30 ± 0.15	0.21 ± 0.12	0.27 ± 0.16	0.29 ± 0.14	0.25 ± 0.12	0.29 ± 0.12	0.25 ± 0.12	0.26 ± 0.16
Insulin (IU/L)	-30	0	20	40	60	90	120	240
Theophylline	9.1 ± 3.6	8.0 ± 2.5	7.9 ± 2.4	7.6 ± 3.2	8.8 ± 3.3	8.9 ± 2.7	8.1 ± 2.6	7.1 ± 1.7
Placebo	8.8 ± 2.6	8.9 ± 2.4	8.3 ± 2.4	7.6 ± 2.1	8.0 ± 2.1	8.0 ± 2.4	8.4 ± 2.0	8.0 ± 1.7
Enprofylline	11.1 ± 4.7	10.8 ± 5.4	11.3 ± 4.1	9.5 ± 3.1	10.0 ± 2.5	10.5 ± 2.8	8.6 ± 3.8	7.4 ± 2.5
Glucagon (ng/L)	-30	0	20	40	60	90	120	240
Theophylline	72.5 ± 19.1	67.0 ± 23.5	62.1 ± 23.6	70.4 ± 31.5	64.0 ± 31.2	64.3 ± 25.5	64.8 ± 24.8	62.9 ± 32.6
Placebo	63.7 ± 26.5	59.4 ± 16.3	57.9 ± 20.3	59.8 ± 17.6	54.3 ± 18.1	54.4 ± 18.9	54.4 ± 20.9	56.4 ± 19.5
Enprofylline	66.2 ± 19.1	70.6 ± 23.9	72.6 ± 27.0	69.4 ± 21.7	67.3 ± 25.1	64.6 ± 22.9	62.4 ± 22.9	59.6 ± 22.5
Glucose (mmol/L)	-30	0	20	40	60	90	120	240
Theophylline	4.4 ± 0.67	4.6 ± 0.50	4.6 ± 0.29	4.6 ± 0.44	4.6 ± 0.28	4.5 ± 0.34	4.6 ± 0.41	4.4 ± 0.37
Placebo	4.4 ± 0.37	4.6 ± 0.65	4.6 ± 0.59	4.5 ± 0.36	4.5 ± 0.42	4.5 ± 0.35	4.4 ± 0.33	4.4 ± 0.34
Enprofylline	4.8 ± 0.90	4.6 ± 0.96	4.6 ± 0.76	4.4 ± 0.34	4.4 ± 0.11	4.7 ± 0.65	4.5 ± 0.64	4.3 ± 0.43

Side effects

Four subjects experienced slight headache after the infusion of enprofylline, and one subject felt dizzy after infusion of theophylline. No side-effects were reported after placebo administration.

DISCUSSION

In the present study theophylline markedly increased the plasma levels of FFA and had a pronounced diuretic effect, but did not concomitantly affect the other metabolic variables studied. These observations are in general agreement with findings in earlier investigations of theophylline and caffeine showing that these xanthines consistently induce FFA-release and natriuresis (1, 6, 9, 37, 39, 42). Enprofylline had no effects on diuresis or on any of the metabolic parameters, whereas this xanthine, in contrast to theophylline, produced a slight but statistically significant decrease in blood pressure and increase in heart rate. These effects on blood pressure and heart rate agree with previous findings with enprofylline in man (23, 24). The maximum plasma concentration of theophylline reached about 50 $\mu\text{mol/L}$, which is in the lower region for recommended therapeutic use in asthma. At the achieved maximum plasma levels of about 10 $\mu\text{mol/L}$ enprofylline is known to produce bronchodilation and, e.g. relaxation of the lower esophageal sphincter (LES) in man (20, 23). A plasma level ratio of 5:1 between theophylline and enprofylline was actually aimed at because this appears to be the approximate ratio for their bronchodilator and LES relaxant potencies (20, 23, 24). Furthermore, the absolute plasma levels were around the

threshold for headache and nausea. These are frequent side-effects of acutely administered xanthines in drug-naive subjects (cf. 18). The slight headache and dizziness occurring in the present study probably have not interfered with the evaluation of the metabolic effects.

Theoretically, theophylline and caffeine could increase plasma CA via antagonism of adenosine which has the ability to depress the release of transmitter substances from autonomic nerves (16). It is further conceivable that raised plasma levels of these amines could be a consequence of the CNS-actions of methyl-xanthines. Release of CA has been suggested to explain various effects of theophylline, including its antiasthmatic actions (e.g. 35, 39). In animals theophylline and caffeine have been shown to stimulate release of CA, particularly adrenaline, from the adrenal medulla (8, 29, 35). However, the drug concentrations used were 10-20 times greater than those used clinically. In man, data on CA release by xanthines are equivocal (1, 7, 8, 9, 12). Vestal et al (40) studying the effect of four different doses of theophylline (aminophylline) found a slowly developing increase of both adrenaline and noradrenaline concentrations in plasma. These increases did not coincide with maximum plasma theophylline concentrations. At a plasma theophylline level of 10 $\mu\text{g/ml}$ (55 μM) plasma adrenaline increased from 30 to 57 pg/ml and plasma noradrenaline from 178 to 244 pg/ml .

The present study shows that theophylline 53 μM or enprofylline 11 μM , which are known to produce bronchodilation and other pharmacological effects in man (20, 23, 24), may not be associated

with changes in plasma CA. These findings do not contradict the possibility that theophylline-induced elevations of glucose and renin (and possibly glucagon and insulin) in plasma, when they occur (cf. 4, 14, 40), are secondary to CA-release; neither variable was changed in this study. On the other hand, contrary to what has been suggested by others (36, 40), our data indicate that lipolysis after theophylline is not necessarily produced via raised plasma levels of catecholamines. Supporting this view, theophylline caused a similar increase in FFA in adrenalectomized and normal subjects (13) ruling out adrenaline release from the adrenal medulla being responsible for this effect. Propranolol attenuated but did not prevent the increase of FFA produced by theophylline in normal subjects (13).

Due to the fact that theophylline is a phosphodiesterase inhibitor and to the possibility that increased cellular levels of cyclic AMP could mediate lipolysis, it has for a long time been considered unobjectionable to hold phosphodiesterase inhibition responsible for the lipolytic action of this xanthine. However, at therapeutic and low plasma levels (as achieved in the present study), theophylline does not seem to inhibit phosphodiesterase enzymes (7). In contrast, already at 10 μ M theophylline antagonizes the antilipolytic actions of adenosine and this nucleotide has recently been suggested to have a role in the regulation of adipose tissue circulation and lipolysis (15, 28). In the present study, a select dose of enprofylline, an alkylxanthine which is a poor adenosine antagonist in a variety of tissues (32, 33) including rat fat cells (17) had no lipolytic action. In accordance with the hypothesis that pharmacodynamic dissimilarities between

theophylline and enprofylline may indicate the importance of adenosine antagonism (32), the present findings support the possibility that an endogenous adenosine-like mechanism down-regulates lipolysis in humans. The clinical importance of these findings are not known but excessive release of FFA may be considered as an undesirable action of xanthine drugs. For example, raised plasma levels have been suggested to play a role in the development of ventricular arrhythmias (25).

The potassium-sparing and potent natriuretic and volume diuretic actions of theophylline were clearly illustrated in the present study (cf. 5, 32) where enprofylline did not show any diuretic action. The results may be compared with findings in a rat study where theophylline from 5 to 20 mg/kg produced a marked natriuresis with little effect on potassium excretion. In contrast, enprofylline 2.5-20 mg/kg was devoid of natriuretic activity (32). Thus animal and human data on renal effects of these xanthines agree and support the possibility that adenosine is involved in important regulatory mechanisms of the kidneys (26). It has been suggested that adenosine may mediate a feedback regulation of glomerular filtration rate that is activated by changes in distal tubule flow or sodium chloride delivery (27). The potent natriuretic effect of theophylline and the lack of diuretic actions of the adenosine non-blocking xanthine, enprofylline, are compatible with this suggestion.

In conclusion, this study showed that in normal man plasma levels of enprofylline and theophylline capable of producing bronchodilation, may not be associated with effects on plasma CA.

Enprofylline was shown to differ from theophylline by not inducing FFA-release and natriuresis. It is suggested that these differences are related to the potent ability of theophylline and the poor ability of enprofylline to antagonize physiologically important actions of adenosine.

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V

RELAXATION OF LOWER ESOPHAGEAL SPHINCTER, AND STIMULATION OF
GASTRIC SECRETION AND DIURESIS BY ANTI-ASTHMATIC XANTHINES.
ROLE OF ADENOSINE ANTAGONISM

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ABSTRACT

The study was designed to obtain information on selected extra-pulmonary effects of the adenosine-non-blocking alkyl-xanthine enprofylline that is about five times more potent than the adenosine receptor antagonist theophylline as a bronchodilator. Effects on lower esophageal sphincter pressure (LESP), gastric secretion, and diuresis of theophylline (5.0 mg/kg intravenously producing about 55 $\mu\text{mol/ml}$ plasma) and of enprofylline (1.5 mg/kg intravenously producing about 11 $\mu\text{mol/ml}$ plasma) were examined in eight healthy volunteers. Enprofylline and theophylline decreased LESP (by 5.0 ± 2.6 mm Hg, mean $\pm$ SD, $p < 0.001$ and by 5.8 ± 2.7 mm Hg, $p < 0.001$, respectively) but only theophylline stimulated gastric secretion (volume $p < 0.01$ and acidity $p < 0.01$) and urine production (volume $p < 0.01$, and sodium chloride excretion $p < 0.01$). Neither xanthine affected plasma gastrin. Enprofylline and theophylline can be expected to have a similar ability to impede the barrier to gastroesophageal reflux but only the latter should have additional stimulant effects on gastric secretion, and diuresis. These findings may have clinical significance and suggest a role for adenosine in regulating diuresis and gastric secretion but not LESP.

Key words: Enprofylline, theophylline, adenosine receptor antagonism, lower esophageal sphincter pressure, gastric secretion, diuresis.

INTRODUCTION

Studies in animals and man have shown that enprofylline (PINN, 3-propylxanthine) is a potent anti-asthmatic xanthine derivative lacking a number of theophylline-like extrapulmonary effects (1,2,3,4,5,6,7,8,9,10) (Fig. 1). The different profiles of action of enprofylline and theophylline have been attributed to differences in adenosine antagonism (11,12). Theophylline is generally a potent and enprofylline a poor adenosine antagonist in various tissues harbouring different subtypes of adenosine receptors (2,3,13,14,15). These observations give increasing support to the working hypothesis proposing that a pharmacodynamic difference between enprofylline and theophylline will indicate a specific role of endogenous adenosine (11). Furthermore, enprofylline undergoes negligible metabolism indicating that effects of metabolites will not interfere with the profile of action of this alkylxanthine (4,16,17).

Theophylline is known to increase gastric secretion (18,19) and decrease lower esophageal pressure (LESP) (20,21) in man, but the subcellular mechanisms involved have not been assessed. Both actions promote gastroesophageal reflux and it has been suggested that repeated reflux of acidic contents may aggravate lung disorders through episodes of aspiration or through activation of reflexes by way of vagal afferent and efferent pathways (21,22).

FIG. 1

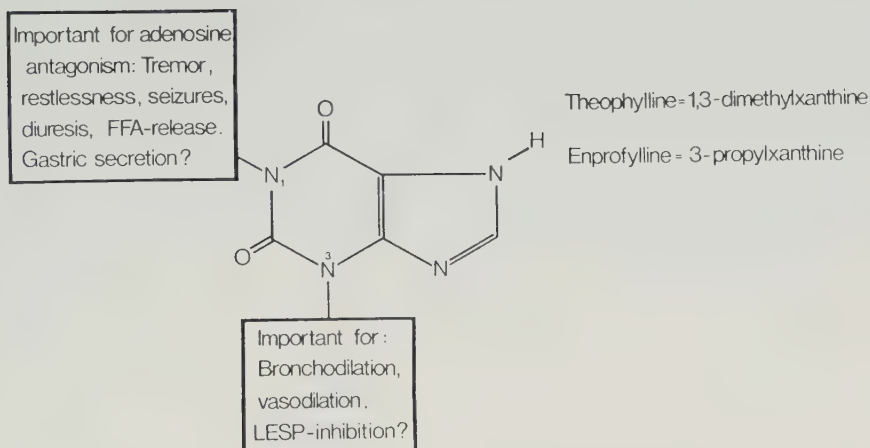


Fig. 1 Substitution at the 1- and 3-positions of the xanthine molecule seems particularly important for the potencies and profiles of action of alkylxanthines (1,3,11,12). Theophylline has a methyl in 1-position which is essential for its potent adenosine receptor antagonism. A variety of extrapulmonary actions apparently reflect this mechanism. By avoiding substitution in the 1- and optimizing alkyl- in the 3-position potent bronchodilating xanthines can be produced (1) that lack adenosine antagonism and that lack many theophylline-like extrapulmonary actions. Furthermore, 3-alkyl-xanthines may depend on renal excretion only for their elimination (1). Enprofylline substantiates these pharmacodynamic and pharmacokinetic relationships.

In this study effects on LESP and gastric secretion of select single doses of theophylline and enprofylline were examined in healthy volunteers. Furthermore, to illustrate one qualitative difference between enprofylline and theophylline the urine production and the urinary excretion of electrolytes were measured concomitantly to effects on LESP. The natriuretic effect of theophylline that once brought this agent into therapeutic use (23) can be explained by adenosine antagonism (24) and, hence, would not be shared by the adenosine non-blocking xanthine, enprofylline (4,10,25). The results have been presented in part earlier (25 b).

METHODS

The trial was performed in two parts each part involving eight healthy male volunteers who gave their written informed consent to participate. The study protocol was approved by the Ethical Committee of the University of Lund. In part I the effects of enprofylline, theophylline, and placebo on LESP and diuresis were compared while the effects on gastric secretion were investigated in part II.

Effects on LESP and diuresis

The mean age ($\pm$ SD) of the subjects was 29 ± 7.1 years, body weight averaged 80 ± 8.3 kg and the mean height was 183 ± 7 cm. The drugs were given on three different days by double-blind, randomized, cross-over technique. The subjects had to abstain

from coffee and xanthine-containing beverages 72 hours prior to their visit to the clinic and had no food or beverages eight hours before their arrival in the morning.

After voiding and drinking 100 ml of water the subjects had a nasogastric three-lumen-tube with 1.1 mm ID and distal side-holes spaced 5 cm apart inserted. Thirty minutes later LESP was measured in supine by three Beckman Pressure Transducers attached to the water-perfused (1 ml/min) tube. A nitrogen pressurized Arndorfer pump was used. Pressure was monitored with 1 cm intervals using the stationary pull through technique (26). End-expiratory values obtained at the respiratory inversion point were considered to represent LESP and the mean of three pressures was calculated.

Registrations were performed before and after 20 minutes' constant infusion of enprofylline (1.5 mg/kg) or theophylline (5.0 mg/kg) or placebo from an infusion pump. Urine was collected until two hours after the infusion and analysed with respect to volume and contents of Na^+ , K^+ , and Cl^- .

Blood samples were taken before, immediately after the infusion, after the second registration of LESP, and two hours after the infusion from an antecubital vein of the arm opposite to the infusion arm and were analysed for content of xanthines and gastrin. Spontaneously reported adverse reactions were registered.

Effects on gastric secretion

The mean age of the subjects was 29 ± 6.7 years, body weight averaged 79 ± 12 kg, and the mean height was 180 ± 4 cm. The

regimens for infusion of xanthines and placebo and for intake of food and beverages were as in part I (above). A 14 French double lumen nasogastric tube was inserted and the gastric content was continuously collected in the supine in eight 15-minute samples using an intermittent suction pump (Egnell, Sweden). The gastric juice was quantified with respect to volume, acidity, Na^+ , K^+ content. During collection of the fifth sample the drugs were given by 15 minutes' constant infusion from an infusion pump.

Blood samples were obtained before, immediately after infusion of drugs, and further three times at 15-minute intervals. The samples were analysed for content of xanthines and gastrin. Spontaneously reported adverse reactions were registered.

Analysis

The xanthines were analysed by an HPLC-method with electrochemical detection which allowed determination down to 0.1 mg/L of either xanthine (17,27). Gastrin was quantified by a standard radioimmunoassay technique (Silab, Sweden). The content of electrolytes in urine and gastric juice was measured by flame photometry. The data were analysed by a two-sided paired t-test and a p-value less than 0.05 was considered as significant. All values are given as mean $\pm$ SD.

RESULTS

Plasma concentrations of xanthines

The plasma levels of the xanthines are shown in figure 2. The mean plasma levels in part I were 55.3 ± 7.2 and 10.4 ± 1.1 $\mu\text{mol/L}$ twenty minutes after the infusion of theophylline and enprofylline, respectively, while the concentrations fifteen minutes after the infusion were 60.5 ± 6.2 and 11.9 ± 2.7 $\mu\text{mol/L}$ in part II.

Gastrin

There were no effects of theophylline or enprofylline on the plasma levels of gastrin (Table 1).

Effects on LESP

Infusion of theophylline and enprofylline resulted in a similar degree of relaxation of LES (fig. 3).

The absolute fall in pressure was 5.0 ± 2.6 ($p < 0.001$) and 5.8 ± 2.7 mm Hg ($p < 0.001$) after enprofylline and theophylline, while the fall in percentage relative to the starting values were 33 ± 17 ($p < 0.001$) and $35 \pm 18\%$ ($p < 0.001$), respectively.

Diuretic effects

Theophylline, but not enprofylline, increased urine production. The increase in urine volume shown in figure 4 after infusion

FIG. 2a

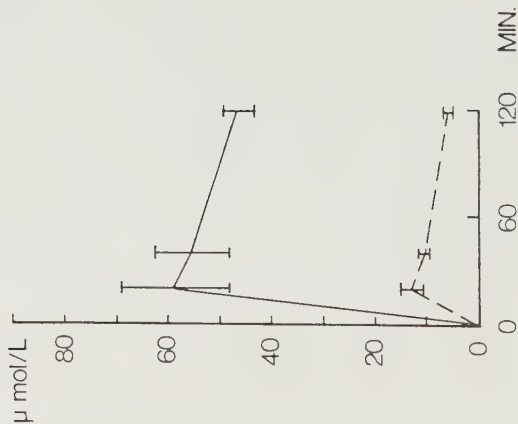


FIG. 2b

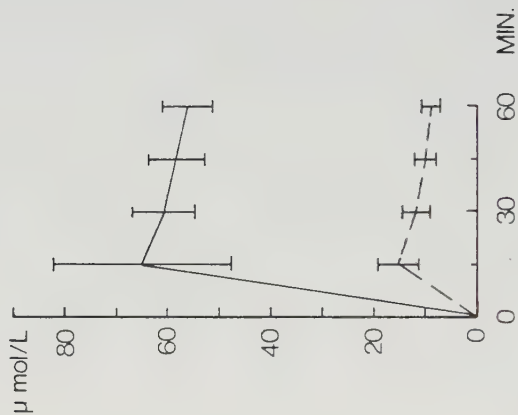


Fig. 2 a) Plasma concentrations (mean $\pm$ SD) of theophylline (—) and enprofylline (---) in subjects where effects on lower esophageal sphincter pressure and diuresis were examined.

b) Plasma concentrations (mean $\pm$ SD) of theophylline (—) and enprofylline (---) in subjects where effects on gastric secretion were examined.

Table 1 Mean $\pm$ SD gastrin plasma levels (pmol/L) before and 0, 15, 30, 45 minutes after completion of the 15 minutes infusion of either drug.

	Before	0	15	30	45
Placebo	30.4 $\pm$ 9.1	29.0 $\pm$ 10.0	31.6 $\pm$ 9.7	32.1 $\pm$ 9.5	29.8 $\pm$ 10.8
Theophylline	25.8 $\pm$ 5.0	32.0 $\pm$ 11.4	27.6 $\pm$ 7.5	32.0 $\pm$ 10.5	33.3 $\pm$ 8.3
Enprofylline	26.3 $\pm$ 6.8	27.9 $\pm$ 6.5	29.1 $\pm$ 5.7	30.0 $\pm$ 8.5	31.3 $\pm$ 9.2

FIG.3

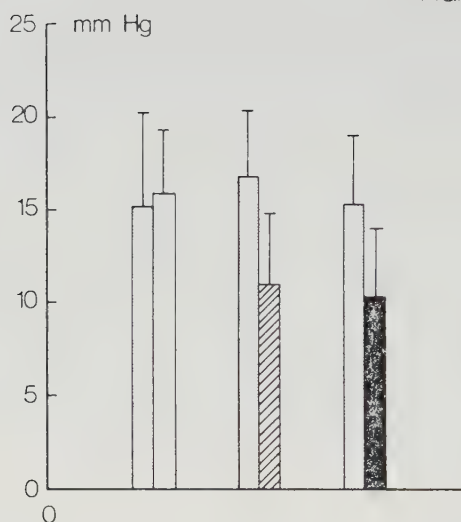


Fig. 3 Effects of placebo (open bar), theophylline (hatched bar), or enprofylline (filled bar) on lower esophageal sphincter pressure (LESP). Shaded bars refer to baseline values. Mean values $\pm$ SD are indicated.

FIG.4

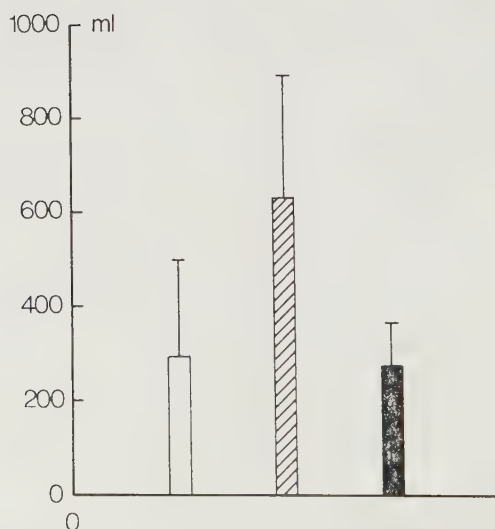


Fig. 4 Mean volume of urine $\pm$ SD collected during 2 hours after infusion of placebo (open bar), theophylline (hatched bar), or enprofylline (filled bar).

FIG. 5a

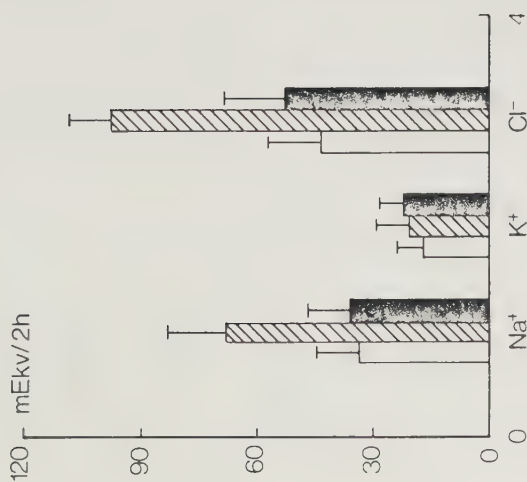


FIG. 5b

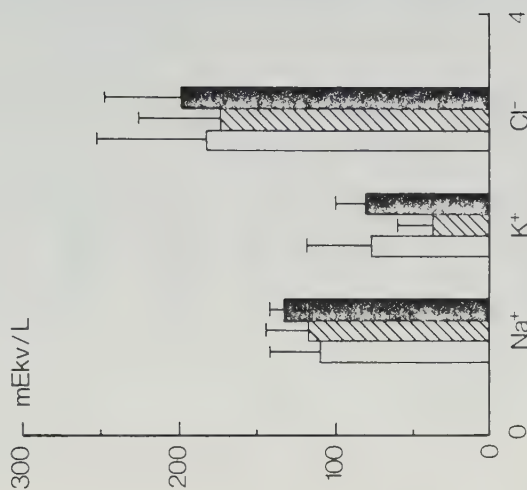


Fig. 5 a) Mean amount of electrolytes excreted ($\pm$ SD) during 2 hours after infusion of placebo (open bars), theophylline (hatched bars) or enprofylline (filled bars).

b) Mean urine concentration ($\pm$ SD) of electrolytes after placebo (open bars), theophylline (hatched bars), or enprofylline (filled bars).

FIG.6

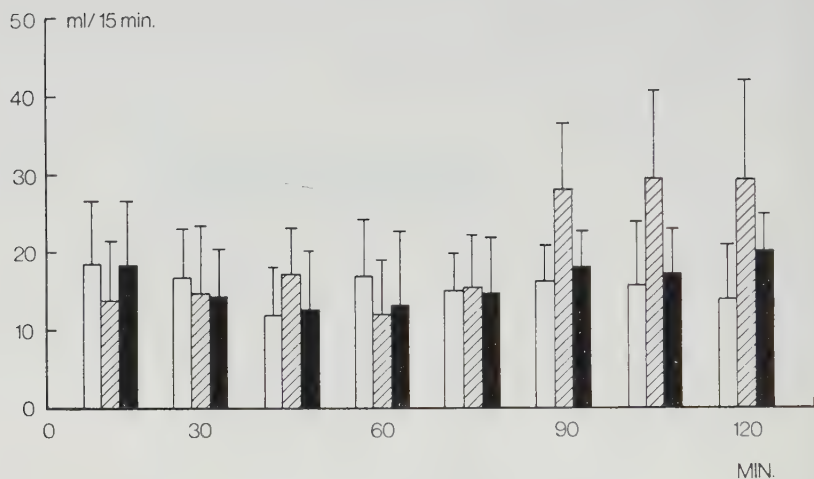


Fig. 6 Effects on volume of gastric secretion (mean values $\pm$ SD) by placebo (open bars), theophylline (hatched bars), and enprofylline (filled bars). The infusions of drugs and placebo were performed during 15 minutes starting at 60 min.

FIG.7

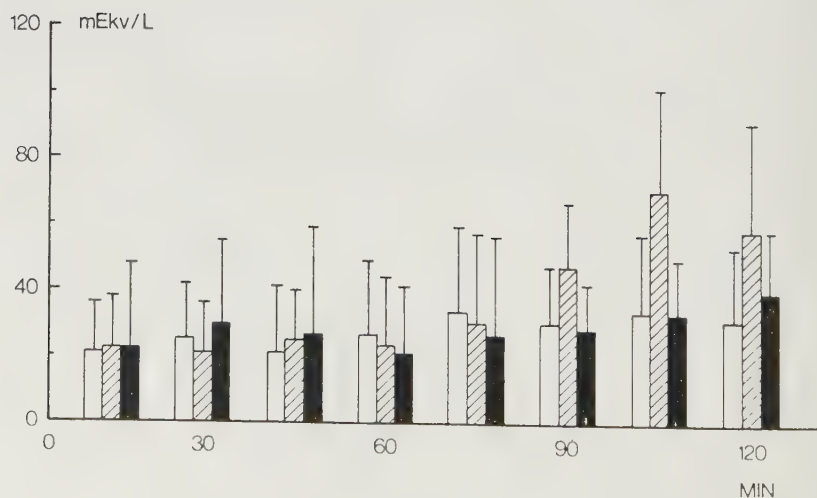


Fig. 7 Effects of placebo (open bars), enprofylline (filled bars), and theophylline (hatched bars) on mean $\pm$ SD gastric H^+ -concentrations in mekv/L as function of time in minutes. The infusions of drugs were performed between 60 and 75 min.

of theophylline was significant ($p < 0.01$) both relative to placebo and enprofylline.

Infusion of theophylline resulted in an increased excretion of sodium and chlorine (fig. 5a). The effects were significant both with respect to placebo ($p < 0.001$) and enprofylline ($p < 0.001$). Theophylline did not affect the amount of excreted potassium (fig. 5a).

There were no effects of theophylline on sodium or chlorine urine concentration while the urine-potassium concentration was reduced both relative to placebo ($p < 0.05$) and enprofylline ($p < 0.001$) (fig. 5b).

Effects on gastric secretion

Theophylline, but not enprofylline, significantly ($p < 0.01$) increased volume of gastric secretion (fig. 6) and only theophylline resulted in an increase ($p < 0.01$) in H^+ -concentration, from 23.9 ± 20.4 mekv/L before infusion to 70.5 ± 31.3 mekv/L 30 minutes after infusion (fig. 7). The amounts of excreted Na^+ , K^+ were significantly ($p < 0.05$) increased from 0.45 ± 0.50 , 0.14 ± 0.15 mekv/15 min before infusion to 0.82 ± 0.33 , 0.38 ± 0.14 mekv/15 min 30 minutes after infusion of theophylline.

Adverse reactions

Part I: Three subjects felt a slight transient pressure in the forehead immediately after infusion of enprofylline.

Part II: One subject experienced nausea, palpitations, urge to void, and a slight flush, while another subject complained of tremor, urge to void, and slight nausea at the end of the infusion of theophylline.

DISCUSSION

While theophylline (and caffeine) are potent stimulants of gastric secretion in man (18,19) animals seem to respond poorly and only at supratherapeutic doses (28,29,30,31). As a consequence of this insensitivity in various experimental in vitro and in vivo systems data are scarce on relevant mechanisms of action operating at the 55 $\mu\text{mol/ml}$ level of theophylline where gastric secretion and acidity was increased in the present study. The effect of theophylline apparently was not due to gastrin release because the plasma levels of gastrin were unchanged.

The present findings actually suggest the possibility of an adenosine mechanism being involved: At plasma concentrations where the xanthines are known to be about equieffective as bronchodilators (6,7,8,9) and as LESP-inhibitors (this study) only theophylline that is a potent adenosine receptor antagonist had the ability to increase gastric secretion and acidity. Although adenosine apparently has not earlier been suggested to be involved in the control of gastric secretion this is theoretically conceivable. Adenosine may at the level of myenteric plexuses in the ventricular wall depress a vagal influence on gastric secretion (cf.3). The possibility of an adenosine involvement in gastric secretion is thus compatible with the

stimulant action of theophylline and the lack of effect of enprofylline. Inferentially, the physiological role of adenosine in the normal regulation of gastric secretion in man should deserve careful examination. A role of adenosine in the regulation of gastric secretion has since been supported by data obtained in a dog study showing that intraarterially administered adenosine inhibited both histamine and methacholine induced gastric secretion (38). The present data also suggest that in contrast to theophylline (18,19) enprofylline may not be contraindicated in patients with an active ulcer.

It has recently been established that theophylline decreases the lower esophageal pressure (LESP) in man and induces gastro-esophageal reflux (20,21). This action may aggravate lung disorders because acidic material may be aspirated or the reflux may activate nervous reflex mechanisms leading to bronchoconstriction (21,22). The present findings showed that, at a plasma concentration ratio of roughly one to five, enprofylline and theophylline produced the same degree of decrement in the LESP. This is probably a result of smooth muscle relaxatory effects and agrees with observations suggesting that enprofylline is about five times more potent than theophylline to relax airway and vascular smooth muscle (2,3,12,14). Neither xanthine affected the plasma gastrin levels indicating that this hormone did not participate in the effects on LESP. The subcellular basis for xanthine-induced smooth muscle relaxant effects is poorly understood. At the achieved plasma concentrations theophylline and enprofylline would have negligible inhibitory effects on phospho-

diesterase enzymes (1,32 and unpublished work by H Bergstrand) which makes the involvement of this mechanism in the LESP-relaxation less likely. The present observation that both an adenosine blocking and an adenosine non-blocking alkylxanthine are effective relaxants suggest that adenosine receptor antagonism also is not involved. This view is supported by a study in opossums showing that adenosine did not constrict the LES (33).

Enprofylline and theophylline can be expected to have a similar ability to impede the barrier to gastric reflux, but only the latter would have the additional stimulant effects on gastric secretion.

A number of observations suggest that adenosine is involved in diuretic actions of alkylxanthines. Adenosine may produce antidiuretic effects by participating in a tubulu-glomerular feedback mechanism that is inhibited by theophylline (34,35). Administration of adenosine to experimental animals has resulted in decreased glomerular filtration rate, decreased urine volume, and decreased sodium excretion (24,34,35). These effects are opposite to and antagonised by theophylline (24). In contrast to theophylline a wide range of doses of enprofylline given to rats were without natriuretic effects, giving further support to the importance of adenosine in the kidneys (2,4). In a previous study involving human volunteers neither the diuretic nor the free fatty acid releasing effects of theophylline were shared by enprofylline (10). The present data confirmed that theophylline, but not enprofylline, had natriuretic and volume-diuretic effects. From an asthma-therapeutic point of view the diuretic activity of xanthines is of doubtful value, except when bronchial asthma

is complicated with cardiac insufficiency (36). Nocturia is listed among side effects of theophylline therapy and diuretic effects would not be desirable in dehydrated patients with acute and severe asthma. It has also been suggested that during treatment of infants with theophylline a hazardous hyponatremia may develop because of its renal actions (37).

In conclusion, the present study in human volunteers has shown that enprofylline and theophylline at a plasma concentration ratio of one to five produced equal relaxant effects on LES. Enprofylline, however, lacked the theophylline-like diuretic and gastric secretory actions. It is suggested that this difference in profile of action is related to a poor ability of enprofylline and a potent ability of theophylline to antagonize physiologically important adenosine actions.

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VI

ABSORPTION OF ENPROFYLLINE FROM THE GASTROINTESTINAL TRACT
IN HEALTHY SUBJECTS

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Kjellin G, Persson CGA, Sjölund K

ABSTRACT

Enprofylline, a new potent bronchodilator xanthine drug, was given orally as a water solution to six healthy subjects in single doses of 2, 4 and 6 mg/kg. The two lower doses produced plasma concentrations in the range 1-4 mg/L, i.e. in the assumed "therapeutic interval" according to previous animal studies. A high 24 h urine recovery of unchanged drug, with mean values for the three dose levels ranging from 85 to 91% of given dose indicated good absorption and little metabolism. The ratio of area under the plasma concentration-time curve/dose increased with dose when the latter increased from 2 to 6 mg/kg. This indicates that the elimination of enprofylline is capacity limited at high doses. Although the drug was given as a solution the mean absorption time (MAT) was prolonged from 0.6 ± 0.1 hours at 2 mg/kg to 1.2 ± 0.1 and 1.6 ± 0.2 hours at 4 and 6 mg/kg, respectively. Multiple peaks in the plasma concentration-time curves at the higher dose levels suggested intermittent and delayed gastric emptying as a possible explanation. This hypothesis was confirmed by studies in six other healthy subjects who received

the drug solution by three different routes; via a catheter in the duodenum, per os, and rectally. The corresponding time to peak values (mean $\pm$ SEM) were 13.3 ± 2.5 , 32.5 ± 8.7 and 157 ± 23 minutes.

Key words: Enprofylline - healthy subjects - absorption - pharmacokinetics.

INTRODUCTION

Enprofylline (3-propylxanthine) has been shown to possess antiasthmatic properties in animals (1) as well as in man (2,3,4). Enprofylline seems to be about five times as potent as theophylline in these actions while it is lacking the central stimulant effect (5,6) and diuretic (5) effects of theophylline. The differences in pharmacodynamic profiles seem to be related to the fact that theophylline, but not enprofylline, is a potent adenosine receptor antagonist (6). Contrary to theophylline, enprofylline has been shown to be metabolized to a very low degree and eliminated almost solely by renal excretion in rats (7) and in man (8). When given orally to rats, enprofylline was completely absorbed (7).

The present study deals with the oral, human pharmacokinetics of enprofylline. The aim of the study was to obtain pharmacokinetic data on enprofylline when given orally as a water solution at three different doses, 2, 4 and 6 mg/kg. The rate and extent of absorption was also compared after 2 mg/kg of the same water solution administered at three different levels in the gastrointestinal channel, namely per os, via a catheter in the duodenum and rectally through a Foley-catheter.

MATERIAL AND METHODS

Study design

The experiments were approved by the ethical committee of the University Hospital of Lund and were performed in two

parts, with two independent panels of male, healthy subjects who gave their written informed consent to participate. The subjects were healthy according to medical history, a physical examination and routine laboratory tests. In part I three different doses were given orally, while in part II a fix dose of a water solution was given orally, in duodenum or rectally. The study was open with a wash-out period of two weeks between administrations.

Part I

Of the eight healthy male volunteers who took part in an i.v. study (8), five completed the present study. Subjects Nos. 4 and 8 in the i.v. study (numbers are according to the i.v. study) refrained from participating in the present study, and subject No. 1 declined to take the highest oral dose. In all cases the decisions were due to personal reasons and not caused by side-effects in the previous experiments. The mean age of the subjects was 27 years (range 23-34 years) and the mean weight was 76 kg (range 64-94 kg). Xanthine-containing food and beverages were not permitted during 12 hours before and 24 hours after ingestion of the drug. The subjects were allowed a light breakfast not later than 1 hour before the start of the trial.

A water solution containing enprofylline (1.3 mg/ml) was given orally in doses of 2, 4 and 6 mg/kg (10.3, 20.6 and 30.9 $\mu\text{mol/kg}$). Blood samples (5 ml) were collected from an

antecubital vein at 10, 20, 30, 40, 60 and 90 minutes and 2, 3, 4, 5, 6, 8 and 10 hours after drug administration. Urine was collected quantitatively up to 24 hours after the dose.

Part II

Six subjects of mean age 38 years (range 32-46 years) and mean weight 72 kg (range 62-90 kg) were included. They abstained from food and beverages 12 hours before and 3 hours after administration of the drug. A water solution containing enprofylline (1.3 mg/ml) was given in the dose of 2 mg/kg at three occasions. On the first occasion enprofylline was administered via a duodenal catheter at the level of ligamentum Treitz, on the second occasion through a Foley catheter rectally to a level 15 cm above ampulla recti and finally on the third occasion orally. Eight hours prior to the rectal administration the subjects administered at home a standard clyster (Klyx^(R)).

Blood samples (5 ml) were collected from an antecubital vein at 20, 40, 60 minutes and 2, 3, 4, 6, 8 and 24 hours after drug administrations for the measurements of plasma levels of enprofylline. In four subjects plasma levels of enprofylline were also determined 5, 10 and 15 minutes after administration. Urine was collected quantitatively up to 24 hours and during 24-36 hours after the dose.

Handling of samples and analysis of enprofylline

Blood samples were drawn via an indwelling catheter (Venflon^(R)). Heparinized tubes (Venoject^(R)) were used, and plasma was prepared by centrifugation. Plasma as well as urine samples were frozen within one hour and stored at -20°C until analysed.

The urine and plasma levels of unchanged enprofylline were determined by liquid column chromatography (8). The sensitivity of the method permitted determination down to 0.1 mg/L. The inter assay precision was always better than 3.5% at 0.4 and 3.0 mg/L, expressed as coefficient of variation.

Adverse reactions

The subjects were regularly questioned about symptoms that could be attributed to the drug.

Data analysis

Mean values $\pm$ SEM are given in the text. The plasma concentration data in part I have been analysed with respect to peak concentrations. The pharmacokinetic information from an i.v. infusion in this panel has been used to also calculate mean absorption time, MAT (9), area under plasma concentration curves, and to construct Wagner-Nelson plots (10). If a one-compartment model can be applied, this plot gives the amount absorbed as function of time. It should be noted that enprofylline has a fairly insignificant distribution phase

lasting about 0.5 hour (8,11). The plasma data in part II have been analysed with respect to time to peak.

The bioavailability after the various administrations has been estimated by urine recovery of enprofylline.

RESULTS

Part I

Oral doses of enprofylline 2, 4 and 6 mg/kg produced peak plasma levels within 3 hours (figure 1). The mean peak levels increased with increasing dose from 2.06 ± 0.12 mg/L to 3.57 ± 0.24 and 5.55 ± 0.43 mg/L. The mean absorption time (MAT) increased with increasing dose from 0.58 ± 0.10 hours, to 1.15 ± 0.17 and 1.60 ± 0.22 hours after 2, 4 and 6 mg/kg respectively (Table 1). An initial rapid increase in plasma drug concentration was followed by a double peak in subjects 1, 2, 3, 5 and 6 after 4 and 6 mg/kg. From the Wagner-Nelson curves (figure 2) it can be seen that the absorption is terminated 1-2 hours after the 2 mg/kg dose but is still continuing 6 hours after the higher doses in subjects 3, 5 and 7. Notches in the curves indicate where a rapid absorption process has been suddenly interrupted. Area under plasma concentration curves ($AUC_{\text{oral}}^{\infty}$) showed a non-linear increase with increasing dose (Table 2). The urine recovery of unmetabolized enprofylline was high, i.e. 90.9 ± 2.0 , 88.8 ± 4.1 and $84.7 \pm 2.6\%$ after 2, 4 and 6 mg/kg, respectively. Urinary recovery of unchanged enprofylline fell

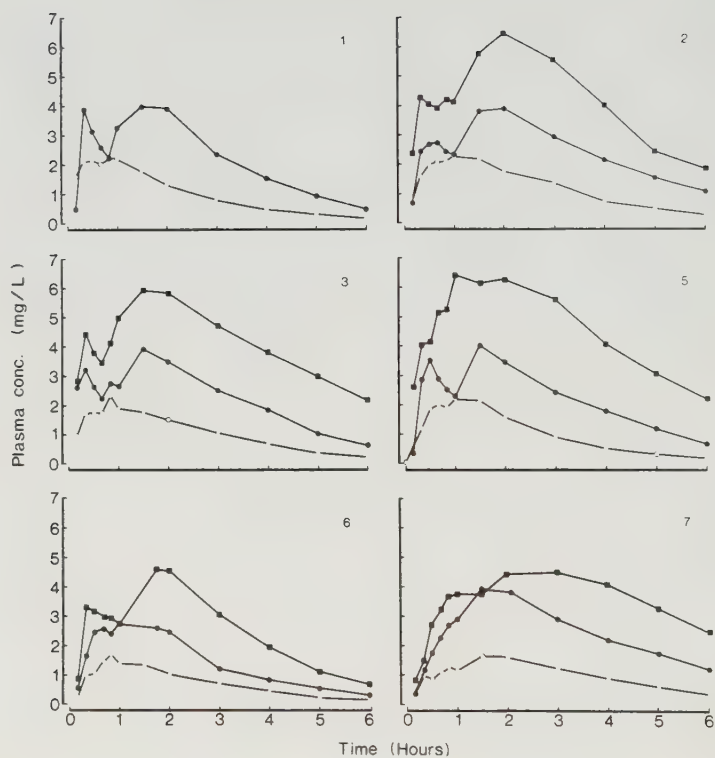


Fig. 1.

Plasma concentration curves in six subjects after oral administration of enprofylline.

○—○ 2 mg/kg; ●—● 4 mg/kg; ■—■ 6 mg/kg

Table 1.

Maximum plasma concentrations ($C_{\max}$) and mean absorption time (MAT) after oral doses of 2, 4 and 6 mg/kg enprofylline

		$C_{\max}$ (mg/L)			MAT (hours)		
Subject No.	Dose mg/kg	2	4	6	2	4	6
1		2.24	3.96	—	0.3	0.8	—
2		2.26	2.89	6.42	0.4	1.3	1.4
3		2.31	3.90	5.90	0.5	0.9	1.6
5		2.20	4.00	6.37	0.7	1.5	1.9
6		1.70	2.76	4.58	0.6	0.7	0.9
7		1.66	3.91	4.46	1.0	1.7	2.2
Mean		2.06	3.57	5.55	0.58	1.15	1.60
SEM		0.12	0.24	0.43	0.10	0.17	0.26

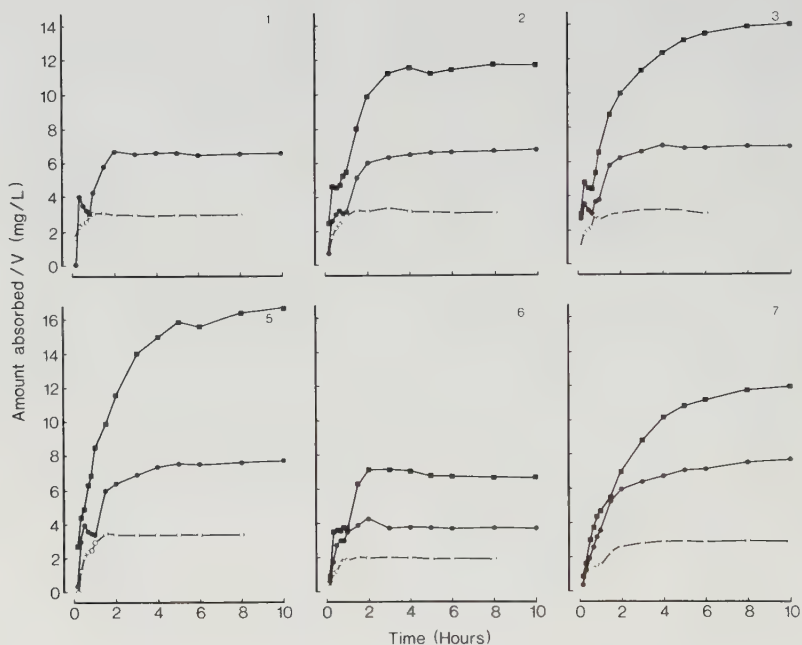


Fig. 2. Rate of drug absorption illustrated by Wagner-Nelson plots of plasma concentration data obtained in six subjects after oral administration of enprofylline. The elimination rate constants were mean values from experiments with three i.v. doses (0.5, 1.0 and 1.5 mg/kg) (8).

○—○ 2 mg/kg; ●—● 4 mg/kg; ■—■ 6 mg/kg

Table 2.

Area under the curve (AUC_{oral}^{∞}) and bioavailability calculated as oral 24 h urine recovery after oral doses of 2, 4 and 6 mg/kg i.v. enprofylline¹⁾

Subject No.	Dose (mg/kg)	AUC_{oral}^{∞} (mg x h/L)			Ratio $\frac{oral}{i.v.}$ urine recovery (%)		
		2	4	6	2	4	6
1		6.38	14.3	—	108	84	—
2		7.91	17.4	30.2	95	98	97
3		6.87	14.9	30.4	101	98	93
5		6.44	14.7	32.5	103	103	95
6		4.79	9.40	16.9	104	106	85
7		7.13	18.4	29.3	106	104	98
Mean		6.59	14.9	27.8	102.8	98.8	93.6
SEM		0.43	1.3	2.8	1.9	3.2	2.3

¹⁾ i.v. data used were obtained from experiments in the same subjects (8)

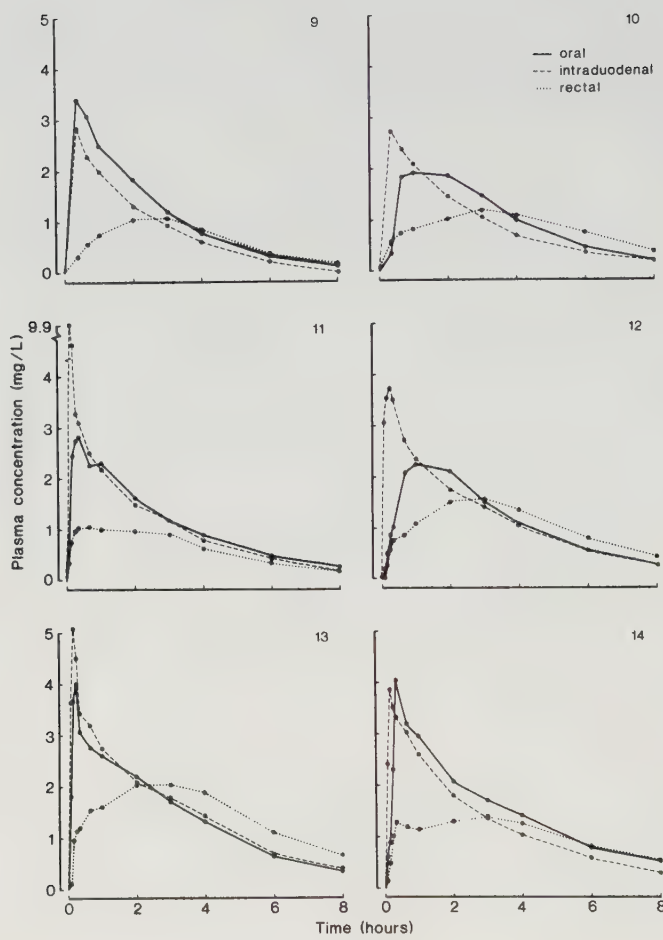


Fig. 3. Plasma concentration curves in six subjects after administration of 2 mg/kg of enprofylline.

slightly from 103% to 94% of that of the i.v. dose when the oral dose increased from 2 to 6 mg/kg.

Adverse effects are reported in reference (12).

Part II

The individual plasma concentrations as a function of time are given in figure 3. No detectable plasma concentrations were obtained 24 hours after administration.

The mean time to peak in plasma concentrations were 13.3 ± 2.47 , 32.5 ± 8.73 and 157 ± 23.4 minutes after duodenal, oral and rectal administration, respectively.

Total individual urine recoveries are given in Table 3. The total urine recoveries of unmetabolized enprofylline were 82.8 ± 5.4 , 82.7 ± 2.0 and 70.2 ± 6.4 percent of given dose after oral, duodenal and rectal administration, respectively. Only marginal amounts of unmetabolized enprofylline were collected in urine between 24 and 36 hours after dosage.

Subjects Nos. 9 and 11 experienced a slight dizziness immediately after duodenal administration while subject No. 11 suffered from nausea and headache after duodenal administration and headache after rectal administration. Subject No. 12 experienced headache in the afternoon after the duodenal administration and subject No. 13 complained about headache after the rectal and the duodenal administration and experienced tremor in the afternoon after the duodenal administration.

Table 3.

Total urine recovery in % of given dose

Subject	Oral	Duodenal	Rectal
9	87.8	87.8	64.9
10	90.1	83.8	79.2
11	87.0	76.5	40.8
12	84.5	77.6	74.2
13	91.0	82.5	79.5
14	56.2	88.0	82.4
Mean	82.8	82.7	70.2
SEM	5.4	2.0	6.4

DISCUSSION

Studies in animals (1) and man (6,11) have indicated that enprofylline is about five times as potent as theophylline in its antiasthmatic actions and a tentative "therapeutic interval" between 1 and 4 mg/L is suggested. The obtained plasma levels in this study have hence been relevant to discuss the oral pharmacokinetic properties of enprofylline at bronchodilating plasma levels in man.

The present data show that enprofylline is completely absorbed when given as an oral solution. However, while the low dose, 2 mg/kg, was rapidly absorbed, the higher dose levels resulted in the occurrence of double or multiple peaks in the plasma concentrations and a prolongation of MAT. The reason for the differences in time dependence of the different oral doses becomes apparent when the data from part II are taken into account. The absorption from duodenum of enprofylline appears to be very fast and consistent, and flat and reproducible plasma concentration curves are detected after rectal administration. However, when given orally to the same subjects varying plasma profiles are obtained with a delayed mean time to peak as compared to duodenal administration. Hence enprofylline seems to be poorly absorbed from the stomach but appears to be very well and consistently absorbed from the small intestines and colon.

The short half-life of enprofylline, around 2 hours (8) will necessitate the use of controlled-release preparations for oral maintenance therapy. With a low rate of release of the

drug, the main part of the absorption will take place in the intestine. Thus, the delayed gastric emptying observed in the present study will probably be of little clinical importance.

The results of the present study indicate capacity limited elimination of enprofylline. This was apparent when the dose was increased from 4 to 6 mg/kg (figure 2, subjects Nos. 2, 3 and 5 and Table 2). The corresponding plasma level range was from 3.5 to 6 mg/L. Further studies in patients are needed to clarify the clinical implications of this dose dependency.

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